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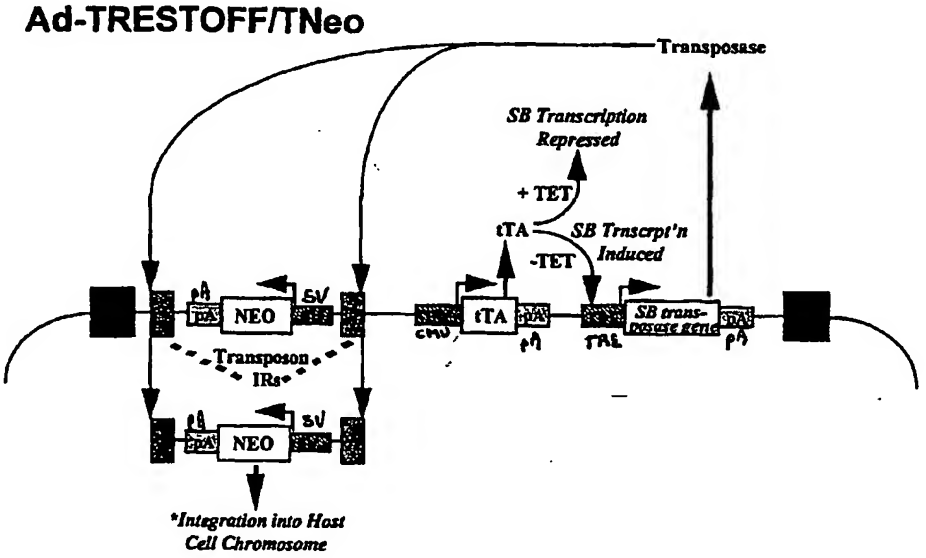

(57) Abstract: The present invention provides non-integrating vectors, preferably non-integrating viral vectors, that include a transposon, preferably a transposon and nucleotide sequences encoding a transposase, and most preferably a transposon, nucleotide sequences encoding a transposase, and nucleotide sequences encoding a polypeptide that regulates expression of the transposase. Also provided are methods of using the non-integrating vectors.

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VECTOR-MEDIATED DELIVERY OF INTEGRATING TRANSPOSON SEQUENCES

Continuing Application Data

This application claims the benefit of U.S. Provisional Application Serial No. 60/133,569, filed May 11, 1999, which is incorporated by reference herein.

Background of the Invention

Vectors are vehicles used for the transfer of genetic material into cells. Vectors can generally be classified as viral and non-viral. To date, viral based vectors have shown much greater efficiency in gene delivery than their non-viral counterparts. Viral vectors can be further divided into those that integrate into the cellular genome and those that remain non-integrated. Integrating viruses provide stable gene transduction that may be passed on to progeny derived from the original target cell. The prototype integrating viral vector is retrovirus. In contrast, non-integrating vectors, whether viral or non-viral, provide only transient transduction, since the gene introduced is either broken down or lost as the cell divides. The prototypes for non-integrating viral vectors are adenovirus or herpes virus based. Most clinical studies to date have used either retrovirus-based (integrating) vectors or adenovirus-based (non-integrating) vectors.

Retrovirus vectors have the advantage of providing long term effects due to their ability to integrate into the host cell genome; however, they are difficult to produce in high titers, most can infect only dividing cells, and they are relatively labile and sensitive to *in vivo* inactivation. As a result, retrovirus vectors have met with limited success when used for gene therapies involving direct *in vivo* gene delivery, and have instead been more successfully employed in *ex vivo* or *in vitro* procedures, typically using explanted cells of the host. Transgenic cells transduced *ex vivo* or *in vitro* can be therapeutically administered to a patient. While potentially useful in therapies involving hematopoetic cells and, to a lesser extent, muscle cells and liver cells, these procedures are not practical for gene therapies involving less accessible target

tissues or organs such as the heart, lung, and brain.

Adenovirus based vectors, in contrast, are easily produced in high titers, can infect dividing and non-dividing cells, and are relatively stable and efficient for in *vivo* delivery. The primary disadvantage of adenoviral vectors has been their limited duration of transgene expression, resulting from the inability of these vectors to replicate or to integrate into the cellular genome. Since transgene expression is transient, adenovirus based vectors must be readministered, and this can be problematical as animals typically develop neutralizing antibodies against the adenovirus capsid proteins. Due to the transient expression of transgenes, the use of adenovirus based vectors has been directed to short-term gene therapy treatments for acute conditions.

Transposons are mobile genetic elements that can integrate a gene into a host genome. Naturally occurring transposons usually include a transposase-encoding gene flanked by cis-acting sequences on the termini of the transposon (Fig. 1). These terminal sequences are recognized by a transposase enzyme, which then excises the entire transposon away from its position in the chromosome and reinserts the transposon elsewhere in the genome ("cut-and-paste" mechanism: Fig. 1). Theoretically, a transposon system could be used to integrate a gene of interest into a host genome by supplying the cell with the gene of interest flanked by the necessary cis-acting sequences, together with a source of functional transposase. However, most transposons are species specific and no functional non-retroviral transposon systems have as yet been discovered in mammals. To date, transposon-mediated integration of a gene of interest into the genome of a mammalian cell has been successfully demonstrated only once (e.g., Ivics et al. *Cell* 91, 501-510 (1997); WO 98/40510). Kelly and Wilson (WO 97/15679), on the other hand, reported successful integration of a transgene into the genome of a mammalian cell using an adenovirus incorporating a retrotransposon system, but when retrotransposons are used, genomic integration cannot take place until the host cell divides, severely limiting the utility of this approach for *in vivo* gene therapies. Until now, there have been no reports of successful modification of a non-integrating viral vector, such as an adenovirus, to possess a transposon-

derived integrative capacity capable of operating in vertebrate cells.

Summary of the Invention

In view of the potential use of nucleic acid delivery systems in, for instance, genome analysis and gene therapy, there is a need for viral vectors that deliver to cells nucleic acids that integrate in the absence of cell division. Moreover, due to the advantages of non-integrating viral vectors like adenovirus and herpes simplex virus, there is a need for non-integrating viral vectors that confer long term expression of a transgene delivered by the vector. The present invention combines the well-known advantages of non-integrating viral vectors with the unique ability of a newly created transposon system, the SB transposase system, to facilitate integration of a heterologous polynucleotide into the genome of a vertebrate, preferably a mammal, including a human, mouse, rat, primate, sheep, cow, and pig. Most preferably a human. The implications of the invention on the future of gene therapy are exciting and far-reaching. For example, the adenovirus-mediated transposon delivery vector makes use of a transposon, not a retrotransposon. When retrotransposons are utilized, target cells must be dividing or induced to divide in order to obtain genomic integration of a polynucleotide supplied by the delivery vector. In contrast, transposon sequences can integrate into genomic DNA whether or not the cell is dividing. The term genomic DNA" is used herein to include both chromosomal DNA and extrachromosomal DNA.

The addition of an integrative capacity to non-integrating viral vectors opens up entirely new treatment possibilities, as these viruses can, for the first time, be used for applications that involve long-term production of a polypeptide. Expression of a polypeptide from a coding sequence that has been delivered to, and stably integrated into, the genomic DNA of a cell in accordance with the invention continues until the cell dies. The long-term expression of a polypeptide from a coding sequence integrated into a cell's genomic DNA is also advantageous as it decreases the necessity of readministering the vector. Further, when the transposon is excised from the vector in the cell, the vector self-destructs and is present in a cell for a shorter

period of time. This is advantageous because it is less likely that innate immune mechanisms and cytotoxicity from expression of viral genes will destroy a cell infected with a vector of the present invention.

The present invention provides a non-integrating vector that includes a polynucleotide flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a regulatory sequence comprising an operator sequence. The transposase can be an SB polypeptide having the amino acid sequence of SEQ ID NO:9, or having an amino acid sequence with at least about 80% identity with SEQ ID NO:9. The inverted repeats can include the nucleotide sequence of SEQ ID NO:7 or SEQ ID NO:8. The inverted repeats can include direct repeats having a nucleotide sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5.

The non-integrating vector of can be a non-integrating plasmid vector. Alternatively, the non-integrating vector of can be a non-integrating viral vector, for instance an adenovirus-based vector that includes iterative terminal repeat sequences that flank the combination of the polynucleotide, the inverted repeats, the transposase-encoding polynucleotide, and the regulatory sequence. The adenovirus-based vector can be a helper-dependent adenovirus vector.

The polynucleotide flanked by the inverted repeats can include a non-coding sequence. Alternatively, the polynucleotide flanked by inverted repeats can include a coding sequence operably linked to a regulatory sequence. The operator sequence can be a *lac* operator or a tetracycline response element.

Optionally, the non-integrating vector can further include a regulatory polypeptide-encoding polynucleotide. When the operator sequence includes a *lac* operator sequence, the regulatory polypeptide-encoding polynucleotide encodes LacI. When the operator sequence includes a tetracycline response element, the regulatory polypeptide-encoding polynucleotide encodes a polypeptide that binds to the tetracycline response element, for instance tTA or rtTA, under the appropriate conditions.

In another aspect, the non-integrating vector can include a polynucleotide flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters

expression of the transposase-encoding polynucleotide. The transposase-encoding polynucleotide can be operably linked to a regulatory sequence that includes an operating sequence.

The present invention provides a method for making a transgenic cell including contacting a cell with a non-integrating vector of the present invention such that the transposase is expressed in the cell to yield a transgenic cell that includes genomic DNA including the polynucleotide flanked by inverted repeats. In some aspects the transposase-encoding polynucleotide can include an operator sequence. When the operator sequence is a *lac* operator, expressing the transposase can include maintaining the absence of a LacI polypeptide in the cell, or adding IPTG to the cell. In some aspects the non-integrating vector further includes a regulatory polypeptide-encoding polynucleotide, and the regulatory polypeptide binds to the operator sequence under the appropriate conditions and regulates expression of the transposase-encoding polynucleotide. For instance, when the operator sequence includes a *lac* operator sequence, the regulatory polypeptide-encoding polynucleotide encodes LacI. When the operator sequence includes a tetracycline response element, the regulatory polypeptide-encoding polynucleotide encodes tTA or rtTA. The cells used in the method can be mammalian cell, for instance human cells. The method can be performed *in vivo*, *ex vivo*, or *in vitro*.

The present invention provides a method for making a transgenic organism including contacting a cell with a non-integrating vector of the present invention such that the transposase is expressed in the cell to yield a transgenic organism that includes genomic DNA including the polynucleotide flanked by inverted repeats.

Other aspects of the invention are directed at methods for altering the phenotype of a cell or an organism including contacting a cell with a non-integrating vector of the present invention such that the transposase is expressed in the cell to yield a transgenic cell or organism including genomic DNA that includes the polynucleotide flanked by the inverted repeats, and determining whether a phenotype of the transgenic cell, the transgenic organism, or its progeny is altered in comparison to a cell or organism that does not include the

polynucleotide flanked by inverted repeats.

In another aspect, the present invention is directed to a method for making a nucleic acid delivery system. The method includes constructing a non-integrating adenovirus-based viral vector, for instance a helper-dependent adenovirus vector, that includes a polynucleotide flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a regulatory sequence that includes an operator sequence, and then packaging the non-integrating adenovirus-based viral vector under conditions such that the transposase is not expressed. The operator sequence can be a *lac* operator, and packaging can include maintaining the presence of LacI such that the LacI inhibits expression of the transposase-encoding polypeptide. The non-integrating vector can further include a regulatory polypeptide-encoding polynucleotide encoding LacI. The operator sequence can be a tetracycline response element, and the non-integrating vector can further comprises a regulatory polypeptide-encoding polynucleotide encoding tTA or rtTA

The present invention is also directed to a method for making a nucleic acid delivery system that includes constructing a non-integrating adenovirus-based viral vector, for instance a helper-dependent adenovirus vector, that includes a polynucleotide flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory-polypeptide encoding polynucleotide that alters expression of the transposase-encoding polynucleotide. The method further includes packaging the non-integrating adenovirus-based viral vector under conditions that repress expression of the transposase. The non-integrating vector can further include an operator sequence operably linked to the transposase-encoding polynucleotide. When the operator sequence includes a *lac* operator sequence, the regulatory polypeptide-encoding polynucleotide encodes LacI such that the LacI inhibits expression of the transposase-encoding polypeptide. When the operator sequence includes a tetracycline response element, the regulatory polypeptide-encoding polynucleotide encodes tTA or rtTA.

The present invention is further directed to a method for treating a mammalian patient, for instance a human, including administering to the patient a non-integrating viral vector. The non-integrating viral vector includes a

therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a second regulatory sequence comprising an operator sequence. In another aspect, the non-integrating viral vector includes a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide. The non-integrating viral vector can be administered to an organ or a tissue of the patient, including, for instance, liver, nervous system, brain, lung, skin, cardiovascular system, heart, hematopoietic system, bone marrow, or muscle. The therapeutic agent can be a polypeptide or an RNA molecule. The patient can have a genetic disease characterized by subnormal production of a polypeptide or RNA.

The present invention is also directed to a method for treating a mammalian patient, for instance a human, the includes explanting cells of the patient, and contacting the explanted cells with a non-integrating viral vector that includes a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a second regulatory sequence including an operator sequence. Alternatively, the explanted cells are contacted with a non-integrating viral vector that includes a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide. The transgenic cells are then implanted into the patient to cause production of the therapeutic agent in the patient. The cells can be hematopoietic stem cells, hepatocytes, myoblasts, fibroblasts, kerotinocytes,

endothelial cells or tumor cells, including cancerous tumor cells. The therapeutic agent can be a polypeptide or an RNA molecule.

Brief Description of the Figures

Figure 1 exemplifies the mechanism of a functional transposon; in this embodiment, the coding sequence for the transposase (Gene X) lies between the cis-acting inverted repeats of the transposon.

Figure 2 is an example of an adenovirus-mediated transposon delivery vector; in this embodiment, both the transposase coding sequence and the polynucleotide to be integrated (*neo*) are present on the same vector and flanked by adenovirus (Ad) iterative terminal repeats (ITR). (pA), polyadenylation sequence; P, promoter; SV, simian virus 40 promoter.

Figure 3 exemplifies cross-over events between pACCMVSB10 and pJM17 in the production of an adenovirus-mediated transposase delivery vector, AdSB10. cmv, human cytomegalovirus promoter; mu, map units; I, II, III, and IV, the sites of PCR primers.

Figure 4 is a Western blot showing Sleeping Beauty" transposase (SB) production from human 293 cells transduced with the adenovirus-mediated transposase delivery vector, AdSB10. kDa, kilodalton.

Figure 5 is a graph showing successful transposition of T/*neo* sequences in HeLa cells treated with the adenovirus-mediated transposase delivery vector, AdSB10, and plasmid pT/*neo*, containing the *neo* gene operably linked to the SV40 early promoter and flanked by the cis-acting elements of the SB transposon system. pSB10, plasmid containing nucleotide sequences encoding the SB transposase.

Figure 6 exemplifies tetracycline-regulated transposition from an adenovirus-mediated transposon delivery vector; panel A illustrates a construct that constitutively expresses an activating protein (tTA) causing transposase expression to be repressed in the *presence* of tetracycline (TET); panel B illustrates a construct that constitutively expresses a recombinant activating protein (rtTA) that has been engineered to have the opposite activity, causing transposase expression to be repressed in the *absence* of tetracycline. Ad ITR,

adenoviral iterative terminal repeats; TRE, tetracycline response element; pA, polyadenylation sequence; SV, simian virus 40 promoter; *neo*, a coding sequence that confers resistance to the neomycin analog drug G418; cmv, human cytomegalovirus promoter.

Figure 7 is a plasmid map of pLPBL1.

Figure 8 is a graph showing TRE-controlled transposase expression and transposon function in response to a tetracycline analog. +Dox and -Dox, presence and absence, respectively, of the tetracycline analog doxycycline.

Figure 9 is a graph showing regulation of transposon function in a cell when the transposase and the transposon are present on the same vector.

Figure 10 (A) is a double-stranded nucleic acid sequence encoding the SB protein (SEQ ID NO:10). Fig. 10(B) is the amino acid sequence (SEQ ID NO:9) of an SB transposase. The major functional domains are highlighted; NLS, a bipartite nuclear localization signal; the boxes marked D and E comprising the DDE domain (Doak, et al., *Proc. Natl. Acad, Sci., USA*, 91, 942-946 (1994)) that catalyzes transposition; DD(34)E box, a catalytic domain containing two invariable aspartic acid residues, D(153) and D(244), and a glutamic acid residue, E(279), the latter two separated by 43 amino acids).

Detailed Description of the Invention

Non-integrating vectors

As used herein, the term "non-integrating vector" refers to a polynucleotide that integrates into a host cells' genomic DNA at a nearly undetectable rate. Preferably, a non-integrating vector is incapable of integrating into a host cells' genomic DNA. In some aspects of the present invention, the non-integrative vectors have an integrative capacity that is conferred by the presence of a transposon; however, it is the transposon that integrates into a host cells' genome, not the non-integrative vector. Transposons are described below. As used herein, the terms "host cell," "target cell," and "cell" are used interchangeably to refer to a eukaryotic cell, preferably a mammalian cell, most preferably a human cell, to which a non-integrating vector of the present invention is to be introduced, or has been introduced. As used herein, the term polynucleotide" refers to a polymeric form of nucleotides of

any length, either ribonucleotides or deoxynucleotides, and includes both double- and single-stranded DNA and RNA. A polynucleotide may include nucleotide sequences having different functions, including for instance coding sequences, and non-coding sequences such as regulatory sequences. Coding sequence, non-coding sequence, and regulatory sequence are defined below. A polynucleotide can be obtained directly from a natural source, or can be prepared with the aid of recombinant, enzymatic, or chemical techniques. A polynucleotide can be linear or circular in topology. A polynucleotide can be, for example, a portion of a vector, such as an expression or cloning vector, or a fragment. Unless otherwise specified, the indefinite article "a" means one or more.

Non-integrating vectors include, but are not limited to, non-integrating plasmid vectors and non-integrating viral vectors. A non-integrating vector can provide for further cloning, i.e., amplification of the polynucleotide. A non-integrating vector can also be used to introduce a transposon element to a host cell. Typically, a non-integrating plasmid vector is capable of replication in a bacterial host, for instance *E. coli*. Typically, a non-integrating viral vector is capable of replication in a eukaryotic cell.

Preferably, the non-integrating vector is a non-integrating viral vector. A non-integrating viral vector includes nucleotide sequences that provide for replication and packaging of the polynucleotide under appropriate conditions. Without intending to be limiting, examples of preferred non-integrating viral vectors include adenovirus, herpes simplex virus (HSV), alphavirus, simian virus 40, picornavirus, and vaccinia virus. Another example of a preferred non-integrating viral vector is adeno-associated virus (AAV), which is non-integrative is the cells of some tissues. Preferably, the non-integrating viral vector is an adenovirus, an HSV, or an AAV, more preferably an adenovirus or an HSV, most preferably, an adenovirus. The advantages of non-integrating viral vectors are numerous and include the ability to produce them in high titers, their stability *in vivo*, and their efficient infection of host cells.

Adenovirus vectors of the present invention include iterative terminal repeats (ITRs), which are necessary for replication of an adenoviral vector

during packaging. A preferred embodiment of the invention makes use of a "helper-dependent" (HD) adenovirus vector (see, e.g., WO 97/15679; Parks et al., *Proc. Natl. Acad. Sci. USA*, 93, 13565-13570 (1996); and Hardy et al., *J. Virol.*, 71, 1842-1849 (1997)). HD adenovirus vectors are also referred to in the art as "gutted" or "gutless" adenovirus vectors. HD adenovirus vectors typically elicit a significantly reduced immune response compared to conventional adenoviruses, and also provide more capacity for insertion of the transposon-related components of the construct. An HD adenovirus vector minimally contains the iterative terminal repeats (ITRs) present in adenovirus.

Optionally, the non-integrating vector can further include a delivery vehicle that can be used for delivery of the vector. For instance, the vector can be enclosed in a liposome, or condensed using nucleic acid condensing agents such as polycationic polypeptides (e.g., polylysine-containing polypeptides or polyethylene-imine). Alternatively, if the vector is a viral vector, the vector can be packaged using methods appropriate for the particular vector. Such methods are known to the art (in the case of adenovirus, see for instance Becker et al., *Methods in Cell Biol.*, 43(Pt A), 161-189 (1994); and Parks et al., *Proc. Natl. Acad. Sci. USA*, 93, 13565-13570 (1996)).

In the present invention, the non-integrating vector, preferably a non-integrating viral vector, can be used to deliver to a host cell in various combinations a transposon, a nucleotide sequence encoding a transposase that mediates excision and insertion of the transposon, and/or a nucleotide sequence encoding a regulatory polypeptide that regulates expression of a transposase. Each of these components is described below. Preferably, the non-integrating vector includes a transposon, more preferably both a transposon and nucleotide sequences encoding a transposase. Most preferably, the non-integrating vector includes a transposon, a nucleotide sequence encoding a transposase, and a nucleotide sequence encoding a regulatory polypeptide.

Transposons

The non-integrating vector of the present invention can include a transposon element, also referred to herein as a "transposon." A transposon

includes a polynucleotide flanked by cis-acting nucleotide sequences on the termini of the transposon. The present invention is not limited to the use of a particular transposon element, as it is expected that the non-integrating vector of the invention can be used to deliver any transposon that is functional in the target cell, without limitation. Preferably, the transposon is able to excise from the non-integrating viral vector and integrate into the cell's genomic DNA, whether the cell is dividing or not. A polynucleotide is flanked by" cis-acting nucleotide sequences if at least one cis-acting nucleotide sequence is positioned 5' to the polynucleotide, and at least one cis-acting nucleotide sequence is positioned 3' to the polynucleotide. Cis-acting nucleotide sequences include at least one inverted repeat (IR) at each end of the transposon, to which a transposase, preferably a member of the SB family of transposases, binds. The SB family of transposases is described in greater detail below.

Each inverted repeat preferably includes one or more direct repeats. The nucleotide sequence of the direct repeat is preferably at least about 80% identical with a consensus direct repeat sequence (SEQ ID NO:1) which is described below. A direct repeat is typically between about 25 and about 35 base pairs in length, preferably about 29 to about 31 base pairs in length. Notwithstanding the above, however, an inverted repeat optionally contains only one direct repeat," in which event the direct repeat is not actually a repeat" but is nonetheless a polynucleotide having at least about 80% identity to a consensus direct repeat sequence as described more fully below.

In some aspects of the invention there are two direct repeats in each inverted repeat sequence. The direct repeats (which number, in this embodiment, four) have similar polynucleotides, as described in more detail below. An inverted repeat on the 5' or left" side of a transposon of this embodiment typically comprises a direct repeat (i.e., a left outer repeat), an intervening region, and a second direct repeat (i.e., a left inner repeat). An inverted repeat on the 3' or right" side of a transposon of this embodiment comprises a direct repeat (i.e., a right inner repeat), an intervening region, and a second direct repeat (i.e., a right outer repeat). Because they are inverted with respect to each other on the transposon, the direct repeats in the 5' inverted repeat of the transposon are in a reverse orientation compared to the direct

repeats in the 3' inverted repeat of the transposon. The intervening region within an inverted repeat is generally at least about 150 base pairs in length, preferably at least about 160 base pairs in length. The intervening region is preferably no greater than about 200 base pairs in length, more preferably no greater than about 180 base pairs in length. The nucleotide sequence of the intervening region of one inverted repeat may or may not be similar to the nucleotide sequence of an intervening region in another inverted repeat.

Most transposons have perfect inverted repeats, whereas the inverted repeats that bind SB protein contain direct repeats that preferably have at least about 80% identity to a consensus direct repeat, preferably about 90% identity to a consensus direct repeat. A preferred consensus direct repeat is 5'-CMSWKKRRGTCRGAAGTTTACATACACTTAAK (SEQ ID NO:1) where M is A or C, S is G or C, W is A or T, K is G or T, and R is G or A. The presumed core binding site of SB protein is nucleotides 3 through 31 of SEQ ID NO:1. Nucleotide identity is defined in the context of a comparison between a direct repeat and SEQ ID NO:1, and is determined by aligning the residues of the two polynucleotides (i.e., the nucleotide sequence of the candidate direct repeat and the nucleotide sequence of SEQ ID NO:1) to optimize the number of identical nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of shared nucleotides, although the nucleotides in each sequence must nonetheless remain in their proper order. A candidate direct repeat is the direct repeat being compared to SEQ ID NO:1. Preferably, two nucleotide sequences are compared using the Blastn program, version 2.0.11, of the BLAST 2 search algorithm, as described by Tatiana, et al. (*FEMS Microbiol Lett*, 174, 247-250 (1999)), and available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including reward for match = 1, penalty for mismatch = -2, open gap penalty = 5, extension gap penalty = 2, gap x_dropoff = 50, expect = 10, wordsize = 11, and filter on. In the comparison of two nucleotide sequences using the BLAST search algorithm, nucleotide identity is referred to as identities."

Examples of direct repeat sequences that bind to SB protein include: a left outer repeat 5'-GTTGAAGTCGGAAGTTTACATACACTTAA-3' (SEQ ID

NO:2); a left inner repeat 5'-CAGTGGGTCAGAAGTTTACATACACTAAG-3' (SEQ ID NO:3); a right inner repeat 5'-AACTCACATACAATTGAAGACTGGGTGAC-3' (SEQ ID NO:4); and a right outer repeat 5'-ATTCCACATACATTTGAAGGCTGAAGTTG-3' (SEQ ID NO:5). As written, the right side direct repeats (SEQ ID NOs:4 and 5) are depicted as they would appear on the transposon, i.e., the nucleotides are in a reverse complement order when compared for identity to the polynucleotide of the left side repeats (SEQ ID NOs:2 and 3). Preferably, the inverted repeat (SEQ ID NO:5) is present on the 3' or right side" of a transposon that comprises two direct repeats in each inverted repeat sequence.

In one embodiment the direct repeat sequence includes at least the following sequence: ACATACAC (SEQ ID NO:6).

One preferred inverted repeat sequence of this invention is SEQ ID NO:7

5'-AGTTGAAGTC GGAAGTTTAC ATACACTTAA GTTGGAGTCA TTAAAACTCG
TTTTTCAACT ACACCACAAA TTTCTTGTTA ACAAACAATA GTTTTGGCAA
GTCAGTTAGG ACATCTACTT TGTGCATGAC ACAAGTCATT TTTCCAACAA
TTGTTTACAG ACAGATTATT TCACTTATAA TTCACTGTAT CACAATTCCA
GTGGGTCAGA AGTTTACATA CACTAA-3'

and another preferred inverted repeat sequence of this invention is SEQ ID NO:8

5'-TTGAGTGTAT GTTAACTTCT GACCCACTGG GAATGTGATG AAAGAAATAA
AAGCTGAAAT GAATCATTCT CTCTACTATT ATTCTGATAT TTCACATTCT
TAAAATAAAG TGGTGATCCT AACTGACCTT AAGACAGGGA ATCTTTACTC
GGATTAAATG TCAGGAATTG TGAAAAAGTG AGTTTAAATG TATTTGGCTA
AGGTGTATGT AAACTTCCGA CTTCAACTG-3'.

The inverted repeat (SEQ ID NO:8) contains the poly(A) signal AATAAA at nucleotides 104-109. This poly(A) signal can be used by a coding sequence present in the transposon to result in addition of a poly(A) tail to an mRNA. The addition of a poly(A) tail to an mRNA typically results in increased stability of that mRNA relative to the same mRNA without the poly(A) tail.

The polynucleotide flanked by the IRs can include a coding sequence or a non-coding sequence. A coding sequence" as used herein refers to a polynucleotide that encodes an RNA and, when placed under the control of appropriate regulatory sequences expresses the encoded RNA. The RNA can be a biologically active RNA, including mRNA or a ribozyme. An mRNA can be translated in the host cell to yield a biologically active polypeptide. The boundaries of a coding region are generally determined by a translation start

codon at its 5' end and a translation stop codon at its 3' end. A "regulatory sequence" is a nucleotide sequence that regulates expression of a coding sequence to which it is operably linked. Nonlimiting examples of regulatory sequences include promoters, enhancers, transcription initiation sites, translation start sites, translation stop sites, transcription terminators, and poly(A) signals. The term operably linked" refers to a juxtaposition of components such that they are in a relationship permitting them to function in their intended manner. A regulatory sequence is operably linked" to a coding region when it is joined in such a way that expression of the coding region is achieved under conditions compatible with the regulatory sequence. For convenience, and for reasons that will become apparent below, a regulatory sequence that is operably linked to a coding sequence that is flanked by IRs is referred to herein as a first regulatory sequence.

A promoter present in a first regulatory sequence operably linked to a coding region can be a promoter that is functional in the target cell, including regulatable promoters such as inducible and repressible promoters. An example of a regulatable promoter is a tissue specific promoter, i.e., a promoter that is not expressed unless the promoter is present in a cell that is part of a particular tissue. A non-limiting example of a tissue specific promoter includes the alpha-1 anti-trypson promoter, which is expressed in liver cells. Optionally, the promoter is a constitutive promoter such as the cytomegalovirus early promoter or the SV40 early promoter.

Transposases

The non-integrating vector of the present invention optionally also includes a coding sequence encoding a transposase. The present invention is not limited to the use of a particular transposase, provided the transposase mediates the excision of a transposon from a non-integrating vector of the present invention and subsequent integration of the transposon into the genomic DNA of a target cell.

A preferred transposase for use in the invention is Sleeping Beauty" transposase, referred to herein as SB transposase or SB polypeptide (Z. Ivics et al. *Cell*, 91, 501-510 (1997); WO 98/40510). SB transposase is able to bind the inverted repeat sequences of SEQ ID NOs:7-8 and direct repeat sequences (SEQ ID NOs:2-5) from a transposon, as well as a consensus direct repeat sequence (SEQ ID NO:1). SB transposase includes, from the amino-terminus moving to the carboxy-terminus, a paired-like domain possibly with a leucine zipper, one

or more nuclear localizing domains (NLS) domains and a catalytic domain including a DD(34)E box and a glycine-rich box, as described in detail in WO 98/40510. The SB family of polypeptides includes the polypeptide having the amino acid sequence of SEQ ID NO:9. Preferably, a member of the SB family of polypeptides also includes polypeptides with an amino acid sequence that shares at least about 80% amino acid identity to SEQ ID NO:9; more preferably, it shares at least about 90% amino acid identity therewith, most preferably, about 95% amino acid identity. Amino acid identity is defined in the context of a comparison between the member of the SB family of polypeptides and SEQ ID NO:9, and is determined by aligning the residues of the two amino acid sequences (i.e., a candidate amino acid sequence and the amino acid sequence of SEQ ID NO:9) to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. A candidate amino acid sequence is the amino acid sequence being compared to an amino acid sequence present in SEQ ID NO:9. A candidate amino acid sequence can be isolated from a natural source, or can be produced using recombinant techniques, or chemically or enzymatically synthesized. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.11, of the BLAST 2 search algorithm, as described by Tatiana, et al. (*FEMS Microbiol Lett.*, 174, 247-250 (1999)), and available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, amino acid identity is referred to as identities." SB polypeptides preferably have a molecular weight range of about 35 kD to about 40 kD on about a 10% SDS-polyacrylamide gel.

The SB polypeptides useful in some aspects of the invention include an active analog or active fragment of SEQ ID NO:9. An active analog or active fragment of an SB polypeptide is one that is able to mediate the excision of a transposon from a non-integrating vector, preferably a non-integrating viral vector. An active analog or active fragment can bind the inverted repeat sequences of SEQ ID NOs:7-8 and direct repeat sequences (SEQ ID NOs:2-5) from a transposon, as well as a consensus direct repeat sequence (SEQ ID NO:1).

Active analogs of an SB polypeptide include polypeptides having amino acid substitutions that do not eliminate the ability to excise a transposon from a non-integrating vector. Substitutes for an amino acid may be selected from other members of the class to which the amino acid belongs. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and tyrosine. Polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, aspartate, and glutamate. The positively charged (basic) amino acids include arginine, lysine, and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Examples of preferred conservative substitutions include Lys for Arg and *vice versa* to maintain a positive charge; Glu for Asp and *vice versa* to maintain a negative charge; Ser for Thr so that a free -OH is maintained; and Gln for Asn to maintain a free NH_2.

Active analogs, as that term is used herein, also include modified polypeptides. Modifications of polypeptides of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acids, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like. Active fragments of a polypeptide include a portion of the polypeptide containing deletions or additions of one or more contiguous or noncontiguous amino acids such that the resulting polypeptide will excise a transposon from a non-integrating vector.

The coding sequence encoding an SB polypeptide can have the nucleotide sequence of SEQ ID NO:10, which encodes the amino acid sequence depicted at SEQ ID NO:9. In addition to the amino acid substitutions discussed above that would necessarily alter the SB-encoding nucleotide sequence, there are other nucleotide sequences encoding an SB polypeptide having the same amino acid sequence as an SB protein such as SEQ ID NO:9, but which take advantage of the degeneracy of the three letter codons used to specify a particular amino acid. The degeneracy of the genetic code is well known to the art and is therefore considered to be part of this disclosure. Further, a particular nucleotide sequence can be modified to employ the codons preferred for a particular cell type. These changes are known to those of ordinary skill in the art and are therefore considered part of this invention.

When a non-integrating vector includes both a transposon and a transposase-encoding polynucleotide, expression of the transposase-encoding

polynucleotide is preferably regulated. This allows the vector to be manipulated, e.g., constructed and propagated, under conditions where expression of the transposase is substantially decreased. Thus, a non-integrating vector that includes a transposase-encoding sequence also preferably includes a regulatory sequence operably linked to the coding sequence encoding the transposase. A regulatory sequence that is operably linked to a coding sequence encoding a transposase is referred to herein as a "second" regulatory sequence, unless noted otherwise. Preferably, the second regulatory sequence includes a promoter that is functional in the target cell. For instance, in some embodiments of the invention, the promoter is a constitutive promoter such as the cytomegalovirus promoter. Preferably, such a promoter is not functional in cells used to construct and/or propagate a non-integrating vector before introduction of the vector to a target cell. For instance, in other embodiments regulatable promoters are preferred. It is expected that minimizing expression of a transposase during manipulation of the vector, for instance during packaging of a non-integrating viral vector, is advantageous as it will allow intact vectors to be produced. In these aspects preferred promoters include, for instance, regulatable promoters such as inducible and repressible promoters. An example of a regulatable promoter is a tissue specific promoter, i.e., a promoter that is not expressed unless the promoter is present in a cell that is part of a particular tissue. A non-limiting example of a tissue specific promoter includes the alpha-1 anti-trypson promoter, which is expressed in liver cells. Other non-limiting examples of regulatable promoters include those induced in the presence of heavy metal ions, elevated temperatures, or hormones. Other methods of regulating expression of the transposase known to the art can also be used. Alternatively, methods of regulating the ability of the transposase to bind to the transposon inverted repeats and mediate excision of the transposon from the vector and into the host cell's genomic DNA can be used.

Optionally and preferably, the second regulatory sequence further includes additional nucleotide sequences that provide for regulation of expression of the operably linked coding sequence. For instance, the second regulatory sequence can further include an operator sequence operably linked to the promoter present in the second regulatory sequence. As used herein, the term "operator sequence" is a nucleotide sequence to which a regulatory polypeptide can bind to alter expression of an operably linked coding sequence. As used herein, the term "regulatory polypeptide" includes polypeptides that bind to a regulatory sequence and either prevent transcription from initiating at a

coding sequence operably linked to the regulatory sequence, or activate transcription initiation from a coding sequence operably linked to the regulatory sequence. Preferably, a regulatory polypeptide binds to an operator sequence.

An example of an operator sequence is a *lac* operator sequence. The *lac* operator is a nucleotide sequence to which the LacI repressor protein binds and prevents transcription initiation. The use of *lac* operators to regulate expression of operably linked coding sequences is known to the art (see, for instance, Beckwith, lac : *The Genetic System*, in: The Operon, Miller et al. (eds.), Cold Spring Harbor Laboratory Press, New York, 11-30 (1980); and Fieck et al., *Nucleic Acids Res.*, 20, 1785-1791 (1992)). A further example of an operator sequence is a tetracycline response element (TRE). The TRE is a nucleotide sequence to which an inducer polypeptide binds under certain conditions to induce expression. The use of TREs to regulate expression of operably linked coding sequences is known to the art (see, for instance, U.S. Patents 5,464,758 (Gossen et al.), and 5,814,618 (Bujard et al.)).

Regulatory Polypeptides

The non-integrating vector of the present invention can further include a coding sequence encoding a regulatory polypeptide. As discussed above, a regulatory polypeptide includes polypeptides that either prevent transcription from initiating at a coding sequence, or activate transcription initiation from a coding sequence. Preferably, the action of the regulatory polypeptide can be modified by the addition of a compound. An example of a regulatory polypeptide includes the LacI repressor, which binds to the *lac* operator in the absence of isopropyl β-D-thiogalactopyranoside (IPTG). Addition of IPTG causes the LacI repressor to no longer bind to the *lac* operator, and thus no longer repress transcription initiation. Preferably, the regulatory polypeptide binds to a region of the second regulatory sequence and affects expression of the transposase.

Additional examples of regulatory polypeptides include tTA and rtTA. tTA binds to a TRE and induces transcription in the absence of tetracycline or an analog thereof. A preferred analog of tetracycline is doxycycline. In the presence of tetracycline or an analog thereof, tTA does not induce transcription. rtTA does not bind to a TRE in the absence of tetracycline or an analog thereof, and thus does not induce transcription of an operably linked coding sequence. In the presence of tetracycline or an analog thereof, rtTA does induce transcription.

A regulatory polypeptide-encoding polynucleotide is preferably operably

linked to a regulatory sequence. Such a regulatory sequence is referred to herein as a "third" regulatory sequence, unless noted otherwise. A third regulatory sequence preferably includes a promoter that is functional in the target cell, including regulatable promoters such as inducible and repressible promoters. An example of a regulatable promoter is a tissue specific promoter, i.e., a promoter that is not expressed unless the promoter is present in a cell that is part of a particular tissue. A non-limiting example of a tissue specific promoter includes the alpha-1 anti-trypson promoter, which is expressed in liver cells. Optionally, the promoter is a constitutive promoter such as the cytomegalovirus early promoter or the SV40 early promoter.

Methods of Use

The present invention is further directed to methods of using the non-integrating vectors, preferably non-integrating viral vectors, described above. In some preferred aspects, methods of using the non-integrating vectors of the present invention are directed to inserting into the genome of a target cell a polynucleotide that is flanked by IRs. Such methods include contacting a cell with a non-integrating vector that includes a polynucleotide flanked by inverted repeats that bind a transposase, and providing the appropriate transposase to the cell to cause the transposon to excise from the vector and insert into the genomic DNA of a target cell. As used herein, the term "contacting" refers to bringing a cell and a vector of the present invention together physically such that the vector enters the cell.

The polynucleotide flanked by inverted repeats can include a coding sequence or a non-coding sequence as described above in the section entitled "*Transposons*." For example, in functional genomics applications, it may be desired to simply disrupt the function of a coding sequence present in the target cell, therefore the polynucleotide that is genomically integrated need not encode an RNA or a polypeptide, and preferably includes nucleotide sequences such as translation stop sites and/or transcription terminators. In other applications, the production of an RNA or polypeptide, particularly a biologically active polypeptide or RNA, in the transgenic cell or organism is intended, and the polynucleotide that is genomically integrated according to the method is selected such that it encodes the desired polypeptide. In other applications the integrated polynucleotide functions as a marker, or encodes a detectable or selectable marker. As used herein, a "marker" refers to a specific nucleotide sequence that can be detected by conventional methods including, for example, hybridization

or the polymerase chain reaction (PCR). As used herein, the terms "detectable marker" and "selectable marker" refer to coding sequences that encode a polypeptide whose presence in a cell can be detected or used to select a cell expressing the marker, respectively. Without intending to be limiting, examples of detectable markers include fluorescent proteins (e.g., green blue, red, yellow), luciferase, and beta-galactosidase. Without intending to be limiting, examples of selectable markers include the polypeptide product of the *neo* gene, which encodes resistance to G418, puro, which encodes resistance to puromycin, mutant dihydrofolate reductase, which encodes resistance to methotrexate.

In one embodiment of the methods, the transposase is provided in trans. For instance, the cell that is contacted with the non-integrating vector that includes a transposon can be further contacted with a second non-integrating vector that includes a coding sequence encoding a transposase. Alternatively, the cell can be contacted with the transposase in a different form. For instance, the cells can be contacted with an mRNA that encodes the transposase, or the cells can be contacted with the transposase polypeptide. In a preferred embodiment of the methods, however, the transposase is provided in cis. For instance, the non-integrating vector that includes the transposon can further contain a coding sequence encoding a transposase. Preferably, the transposase is a member of the SB family of transposases, or an active analog or active fragment thereof, as described above. The coding sequence encoding the transposase can be operably linked to a promoter such that the transposase is expressed when the vector enters the cell.

Alternatively and preferably, as discussed above in the section entitled "*Transposases*" the coding sequence encoding a transposase is operably linked to a regulatory sequence, which can include a promoter as well as an operator sequence. In those aspects of the present invention that include contacting a cell with a non-integrating vector that includes a transposon and a transposase-encoding polynucleotide operably linked to an operator sequence, preferably the non-integrating vector further includes a regulatory polypeptide-encoding polynucleotide. Regulatory polypeptide-encoding polynucleotides are described above in the section entitled "*Regulatory Polypeptides.*"

Preferably, the regulatory polypeptide is expressed when the vector enters the cell, and alters expression of the transposase. The action of the regulatory polypeptide can be modified by the addition or subtraction of a compound. For example, when the regulatory polypeptide is a LacI repressor, the transposase is not expressed unless LacI is prevented from binding to the *lac*

operator sequence. IPTG can be used to prevent LacI from binding to the *lac* operator sequence. When the regulatory polypeptide is tTA or rtTA, the regulatory polypeptide must bind to the tetracycline response element to cause expression of the transposase. Tetracycline or an analog thereof can be used to modify the binding of these regulatory polypeptides to the tetracycline response element. Modifying the action of the regulatory polypeptide results in expression of the transposase and the subsequent excision of the transposon from the vector and integration into the target cell's genomic DNA.

When a cell is contacted with a non-integrating vector of the present invention, preferably the vector is in a form that will allow the vector to enter the cell. For instance, the non-integrating vector can be introduced as naked DNA using methods known to the art. Alternatively and preferably, the non-integrating vector can be enclosed in a delivery vehicle such as a liposome or a nucleoc acid condensing agent. Alternatively, if the vector is a viral vector, preferably an adenoviral vector, the vector can be packaged using methods appropriate for the particular vector.

A cell that is contacted with a non-integrating vector of the present invention can be *in vitro* (i.e., in cell culture), *ex vivo* (i.e., a cell that has been removed from the body of a subject), or *in vivo* (i.e., within the body of a subject). Preferably, the cell is *in vitro*, *ex vivo*, or *in vivo*, more preferably *in vitro*, or *ex vivo*, most preferably *in vitro*. The invention is not intended to be limited by the type of cell that is contacted, for instance the non-integrating vectors of the present invention can be introduced to cells that have been isolated from, or are present in, any organ, tissue, or bodily fluid.

The insertion into a target cell's genome of a transposon using the non-integrating vectors of the present invention can be used to yield a transgenic cell. As used herein, the term "transgenic" includes a cell that contains a transposon delivered by the non-integrating vectors of the present invention, whether the transposon includes a nucleotide sequence that is endogenous to the cell or not. For instance, if the host cell is human, and the transposon includes a human adenosine deaminase-encoding polynucleotide, the host cell is considered to be transgenic.

Optionally, the methods of the present invention can include altering a phenotype of a cell. In this aspect of the invention, a transgenic cell is produced and then observed to determine if a phenotype of the transgenic cell is altered in comparison to a cell that does not contain the transposon. An altered phenotype can be detected by methods known to the art.

In other aspects of the present invention, the methods can be used to produce a transgenic organism that carries a particular marker sequence or expresses a particular polypeptide in one or more cells. Methods for producing transgenic animals are known in the art and the incorporation of the methods of the present invention into these techniques does not require undue experimentation. For instance, mouse embryonic stem cells can be contacted with the non-integrating vectors of the present invention. The transgenic animal can be a mosaic, or all the cells of the animal can contain the transposon. In one aspect, the polypeptide encoded by a coding sequence in the transposon is a product for isolation from a cell. For instance, the transgenic animal can be used as a bioreactor to produce a polypeptide in quantity in milk, urine, blood, eggs or other source easily obtained from the animal.

Optionally, the methods of the present invention can include altering a phenotype of an animal. In this aspect of the invention, a transgenic cell is produced and then used to produce a transgenic animal. The resulting transgenic animal is observed to determine if a phenotype of the transgenic cell is altered in comparison to a cell that does not contain the transposon. An altered phenotype can be detected by methods known to the art.

Other aspects of the present invention are directed to treating an animal by causing the animal's cells to produce a therapeutic agent. These methods can include treating an animal *in vivo* by administering to the animal a non-integrating vector, more preferably a non-integrating viral vector, most preferably an adenovirus, of the present invention. The non-integrating vector includes a transposon that includes a therapeutic agent-encoding polynucleotide. The non-integrating viral vector can be administered or targeted to an organ or a tissue of an animal, including for instance, the liver, nervous system, brain, lung, skin, cardiovascular system, heart, hematopoietic system, bone marrow, and muscle. Delivery of the non-integrating vector can take any conventional form, without limitation other than those imposed by its intended purpose or target. For example, the vector can be delivered by intravenous, intramuscular, subcutaneous, intrathecal or intracranial injection or infusion, or by inhalation. Successful *in vivo* transduction of lung epithelial cells via inhalation using a conventional adenovirus has been demonstrated (Bellon et al., *Human Gene Ther.*, 8, 15-25 (1997)). Methods for targeting the vector a specific type of cell can be used if necessary. Such methods are known to the art. Optionally and preferably, the presence of a compound, for instance IPTG or tetracycline or an analog thereof, is altered in the animal such that the transposase is expressed and

the transposon excises from the non-integrating vector and inserts into the genomic DNA of those cells containing the vector.

In addition to direct introduction of the therapeutic nucleic acid *in vivo*, gene therapies of the invention include transduction of cells *ex vivo* followed by administration of the resulting transgenic cells to the patient. *Ex vivo* applications contemplate using either cells that were explanted from the patient, or other cells, and may include transduction and implantation of cells that are explanted from a different species than the patient. In this aspect of the invention, cells are explanted from the patient and contacted with a non-integrating vector, more preferably a non-integrating viral vector, most preferably an adenovirus, of the present invention. The non-integrating vector includes a transposon that includes a therapeutic agent-encoding polynucleotide. Explanted cells contacted with the vector are then implanted into the animal to cause production of the therapeutic agent in the animal. Non-limiting examples of cells that can be used include hematopoietic stem cells, hepatocytes, myoblasts, fibroblasts, kerotinocytes, endothelial cells, tumor cells and cancerous tumor cells.

The therapeutic agent that is encoded by the transposon can be a polypeptide or an RNA molecule. The methods of treatment of the present invention are useful in treatment of genetic disease, involving for instance replacement of a defective gene, delivery of a polypeptide drug, or supplementation of a metabolic activity. Cystic fibrosis, diabetes, cardiovascular disease, cancer, and brain malfunction are examples of conditions within reach of treatment using the non-integrating vectors of the invention. For example, an adenovirus vector of the present invention can be used to integrate a functional CFTR coding sequence into the lungs of a patient affected with cystic fibrosis; likewise, an adenovirus vector of the present invention can be used to deliver a polynucleotide encoding tissue plasminogen activator (TPA) to cardiac cells of heart disease patients in order to augment low levels of TPA.

To treat cancer, a non-integrating vector of the present invention can be used for the *in vivo* introduction into the genome of a normal cell or a cancer cell a coding sequence encoding a polypeptide that augments, supplements or elicits the patient's immune response. For example, the mammalian cell can be caused to secrete one or more cytokines or to express surface molecules that attract cells of the immune system. In another application, the non-integrating vector of the present invention can be used to create transgenic host cells that provide normal cells with protection against toxic side effects of chemotherapy, by, for example,

expressing a coding sequence that confers resistance to the drug. Another approach involves activation of a prodrug. For example, the adenovirus vector of the present invention can be used to create transgenic cancer cells in the patient engineered to express thymidine kinase from herpes simplex virus (HSV-tk). When a nucleotide analog, such as ganciclovir or acyclovir, is administered, it is incorporated into the nucleic acid of the transgenic cell, causing it to die. Optionally the non-integrating vector includes not only a transposon-competent HSV-tk coding sequence intended to be integrated into the host cell's genome, but also an HSV-tk coding sequence that is constitutively expressed from the adenovirus, for additional short term efficacy.

The presence of a transposon of the present invention in the genomic DNA of a target cell can be verified by several methods known to the art. For instance, if the integrated polynucleotide functions as a marker, the marker can be detected by hybridization or by PCR. If the integrated polynucleotide encodes a detectable or selectable marker, the marker can be detected.

An additional aspect of the present invention is directed to making a transposon delivery system. Making the transposon delivery system includes constructing a non-integrating viral vector that includes, in various combinations, a transposon (described above in the section entitled "*Transposons*"), a transposase-encoding polynucleotide (described above in the section entitled "*Transposases*"), and/or a regulatory polypeptide-encoding polynucleotide (described above in the section entitled "*Regulatory Polypeptides*"). Preferably, the non-integrating vector includes a transposon, more preferably both a transposon and a transposase-encoding polynucleotide. Most preferably, the non-integrating vector includes a transposon, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide.

Making a transposon delivery system also includes packaging the non-integrating viral vector. Packaging methods vary depending on the type of viral vector used and are known to the art. It is expected that expression of the transposase during packaging results in excision of the transposon from the non-integrating viral vector and a decreased efficiency of packaging. Preferably, in those aspects of the present invention where the viral vector includes both transposon and nucleotide sequences encoding a transposase, the non-integrating viral vector is packaged under conditions such that the transposase is not expressed.

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

Example 1

Construction of AdSB10, an SB transposase-transducing adenovirus

To test for adenovirus-mediated delivery of transposase function, an adenovirus vector which transduces the SB10 transposase coding sequence has been constructed by homologous insertion of the SB10 sequence into the adenovirus genome (Figure 3), and packaged. The SB10 transposase coding sequence was removed from pSB10 by digestion with BamHI and SalI. The construction of pSB10 is detailed in Ivics et al. (*Cell* 91, 501-510 (1997)). The BamHI/SalI fragment containing the SB10 transposase coding sequence was ligated between the BamHI and SalI sites downstream of a cytomegalovirus early promoter sequence in pACCMV.pLpA (Becker et al., *Methods in Cell Biol.*, 43(Pt A), 161-189 (1994); Gluzman et al., *Eucaryotic Viral Vectors*, Gluzman, (ed.) pp 187-192, Cold Spring Harbor Laboratory Press, New York (1982)) to form pACCMVSB10. pACCMVSB10 contains adenovirus sequences spanning the region from map units (mu) 0 to 17, interrupted in the E1 region between mu 1.3 and 9.3 by the promoter and transgene sequences.

pACCMVSB10 was co-transfected along with pJM17 into human 293 cells using standard methods (see, for instance, Larregina et al., *Gene Ther.*, 5, 563-568 (1998)). pJM17 (McGrory et al., *Virol.*,163, 614-617 (1988)) contains the entire adenovirus genome interrupted by plasmid sequences at mu 3.7. During homologous recombination (see crossovers, Figure 3), sequences from the pACCMVSB10 construct containing the promoter and transgene replace the region containing the insert at mu 3.7, generating an adenovirus genome containing the cmv early promoter and SB10 coding sequence inserted between mu 1.3 and 9.3 in the adenovirus genome.

Distinct cytopathology (due to spread of recombinant virus) was observed in the cells within 10 days, and supernatants were collected for plaque purification. Plaque purified virus was then expanded for large-scale virus preparation. A Southern blot of the virus probed for the SB10 insert was consistent with homologous insertion into the adenovirus genome. PCR analysis was also conducted to verify presence of the SB10 insert (primers I and II: 5'-CCGCGTTCCGGGTCAAAGTTGGCG (SEQ ID NO:11) and 5' -

GTCACATCCAGCATCACAGGC (SEQ ID NO:12)) as well as homologous recombination between the pACCMVSB10 plasmid and the adenovirus genome (primers III and IV (5' - GGAAGGCTACCCGAAACGTTT (SEQ ID NO:13) and 5' - CCAAGTTGCTGTCCAACGCC (SEQ ID NO:14)). These results were consistent with the successful packaging of pACCMVSB10. The packaged pACCMVSB10 was designated AdSB10.

Example 2

Intracellular expression of the transposase SB by AdSB10

To test for the ability of AdSB10 to express SB transposase protein, extracts of 293 cells infected with the virus were subjected to western blot analysis. The protein extracts were fractionated by SDS-polyacrylamide gel electrophoresis and then electroblotted onto a nitrocellulose support. The blot was then probed for SB protein using a polyclonal antibody prepared against a C-terminal peptide of the SB protein predicted from the SB protein coding sequence. The results clearly show a distinct band (SB arrow) of SB immunoreactive material in the lane loaded with extracts from AdSB10 infected 293 cells which is not present in the control (293 cells) lane (Figure 4). These results demonstrate that transduction of 293 cells with AdSB10 results in expression of SB immunoreactive material in the target cell.

Example 3

Intracellular function of the transposase SB expressed by AdSB10

HeLa cells were transduced to test the function of AdSB10-generated SB transposase. Upon transduction of the purified vector into HeLa cells, the adenovirus vector mediates efficient binding to the cell surface and internalization, followed by endosomal release of the virion capsid, and then transit to the nucleus where the genome is deposited. Once the adenovirus vector genome has been delivered to the nucleus, the CMV promoter mediates expression of SB transposase enzyme.

HeLa cells were transduced with AdSB10 either one day before ((Figure 5, bar 4), one day after (bar 3) or two days after (bar 2) transfection with pT/Neo. Co-transfection of pT/*neo* with pSB10 (bar 5) served as a positive control. pT/*neo* contains a *neo* coding sequence operably linked to an SV40 promoter/enhancer and an SV40 polyadenylation signal, all of which are flanked by inverted repeats. The construction of pT/*neo* is detailed in Ivics et al. (*Cell* 91, 501-510 (1997)). All cultures were plated into selective medium containing

G418 on Day 4. pSB10 co-transfection (bar 5) resulted in a 13.2-fold increase in G418 colony formation in comparison with cells transfected with pT/*neo* alone (bar 1). AdSB10 infection caused a 2.4- to 6.4-fold increase in the frequency of G418-resistant colony formation, indicative that SB10 transposase expressed from the infecting AdSB10 resulted in transposition of T/*neo* sequences. The highest transposition frequency mediated by AdSB10-encoded SB (bar 4) was observed in cells tranduced with AdSB10 prior to transfection with the pT/*neo* transposon. These results demonstrate that the SB10 transposase protein expressed in cells transduced with AdSB10 was capable of carrying out transposition, and inserting new genetic material into the chromosomes of target cells.

Example 4

Generation of AdT/Neo, a *neo* transposon-transducing adenovirus

AdT/*neo* is constructed and packaged in order to assess whether it is possible for transposition to occur from an adenovirus genome to the cellular genome. AdT/*neo* is an adenovirus which contains a transposon containing the *neo* gene as a selectable marker. The *neo* transcription unit along with flanking sequences including an SV40 early promoter and inverted repeat sequences (Fig. 2) is ligated into the pACCMV.pLpA plasmid (Fig. 3) as a KpnI - SalI fragment and is then co-transfected along with pJM17 into human 293 cells as described above for packaging of AdSB10 (Example 1). About 19 days after co-transfection, the cell monolayer is completely lysed. Following this, the medium is collected and any intact cells are lysed by several freeze-thaw cycles. Cell debris are pelleted by centrifugation and the supernatant containing virus is used to infect 293 cells. Following cytopathology, viral DNA is extracted from the cells using the established Hirt method (Hirt, *J. Mol. Biology.*, 26, 365-369 (1967)).

Example 5

Testing for transposition from an adenovirus genome to the host cell genome

The AdT/*neo* vector described in Example 4 may be used in conjunction with a plasmid expressing SB10 transposase to demonstrate transposition in cultured mammalian cells. In this experiment, HeLa cells are transfected with pSB10 either before or after infection with the AdT/*neo* vector. The expression of the SB transposase excises the SV-*neo* transposon from the adenovirus vector

genome and integrates it into the genome of the host cell. Presence of this new genetic material can be tested by colony-formation in the presence of the neomycin analog G418. Efficiency of the process is determined by exposing the host cells to vector at varying concentrations to ensure that all cells have been transduced, and then after 2 days plating the cells at 10-fold serial dilution into selective medium containing G418. Stable introduction of *neo* sequences by transposition is verified by Southern analysis of DNA extracted from G418-resistant clonal populations, verifying the absence of vector sequences and the presence of chromosomal sequences flanking the transposon.

Two days after infection, the HeLa cells are subcultured into selective medium containing G418, and drug-resistant colony formation is scored after two weeks. An increase in the frequency of drug-resistant colony formation in cells transfected with pSB10 in comparison with untransfected cells results from SB transposase-mediated excision of the *neo* expression cassette from the adenovirus vector genome with subsequent transposition and insertion of the *neo* expression cassette into the host cell genome. Further characterization of the sequences flanking the *neo* expression cassette in the cellular genome (using inverse PCR or linker-mediated PCR techniques) verifies that the *neo* sequence is inserted by transposition. Evidence of transposition in these experiments demonstrates that SB transposase is capable of mediating transposition from an adenovirus vector genome as a substrate, with excision of transposon sequences from the adenovirus genome and subsequent transposition with insertion of transposon sequences into the host cell genome.

In a test for independent vector-mediated delivery of both functions, HeLa cells are infected either simultaneously or in succession with AdSB10 for vector-mediated delivery of transposase function, and with AdT/*neo* for vector-mediated delivery of transposon function. Two days later, the cells are plated into selective medium containing G418 and drug-resistant colonies are scored after two weeks. An increase in the frequency of drug-resistant colony formation brought about by infection with AdSB10 in comparison with infection by AdT/*neo* alone is interpreted as evidence for transposition of the *neo* expression cassette from the AdT/*neo* genome to the host cell chromosome, in this case mediated by SB transposase expressed from the AdSB10 vector. Molecular characterization of sequences flanking the integrated *neo* cassette serve to verify that gene transfer occurred by transposition. Evidence for transposition expected in this experiment demonstrates that both transposon and transposase components, delivered from different vectors, function to mediate

transposition from the vector genome to the host cell genome.

Example 6

Generation of adenovirus vectors containing the transposase coding region and the T/*neo* transposon

It is expected that the most effective vector for vector-mediated delivery of transposon sequences is a vector that contains both the transposon and sequences encoding the transposase. However, during the packaging process of an adenovirus containing transposon and sequences encoding the transposase, it is expected that expression of transposase could result in transposon excision and subsequent interruption of the packaging process. This problem is overcome by regulating transposase expression during packaging.

Regulation of transposase expression by tetracycline

Tetracycline regulation of transposition from a single vector will require the introduction of three functions: (i) transposon function; (ii) transposase function under transcriptional regulation of a tetracycline response element (TRE); and (iii) a transcription unit for a tetracycline transactivator, which binds to TRE and regulates expression of SB transposase in response to the presence or absence of tetracycline (Fig. 6). When regulating transposase expression by using a TRE, transposase is not expressed unless a tetracycline transactivator is co-expressed in the same cell. Furthermore, this tetracycline transactivator is either provided in a form in which binding to TRE is activated by tetracycline (termed rtTA or tet-ON) (Gossen et al., *Science*, 268, 1766-1769 (1995)) or in a form which is inhibited by tetracycline (termed tTA or tet-OFF) (Gossen et al., *Proc. Natl. Acad. Sci. USA*, 89, 5547-5551 (1992)). A transcription unit encoding tet-ON or tet-OFF is included in the vector construct along with the transposon and SB transposase functions (Fig. 6).

Packaging of the tet-OFF - containing vector (Fig. 6a) in the presence of tetracycline (or a tetracycline analog) represses SB transposase expression, thus allowing intact packaging of the entire 3-component vector. Subsequent use of the vector to transduce target cells in the absence of tetracycline allows activation of TRE-regulated SB transposase expression, thus allowing SB-mediated transposition to ensue in the transduced target cells.

Packaging of the tet-ON - containing vector (Fig. 6b) in the absence of tetracycline (or a tetracycline analog) also represses SB transposase expression, allowing intact packaging of this 3-component system. Subsequent use of the vector to transduce target cells in the presence of tetracycline activates

TRE-regulated SB-transposase expression, thus allowing SB-mediated transposition to ensue in the target cells.

Regulation of transposase expression and transposon function in a cell

If tetracycline regulation of transposition is to be used to control transposition of the transposon, a TRE-controlled transposase must be able to regulate transposase expression and transposon function in response to tetracycline. This ability was tested (Fig. 8). The plasmid containing the transposon (pT/*neo*) was described by Ivics et al. (*Cell* 91, 501-510 (1997)). ppATRES is a plasmid containing a TRE-regulated SB transposase expression cassette. ppATRES is identical to pTRES, which is described below. The TRE-regulated SB transposase expression cassette was constructed by removing the SB transposase coding sequences from pSB10 and inserting them into the multiple coning site of pTRE (Clontech). The expression cassette was then introduced into the XhoI site of the pLPBL1 adenovirus vector plasmid (Figure 7), and the resulting construct was termed ppATRES. ppATRESTOFF, a derivative of ppATRES that contains the tet-OFF transcription unit (Clontech, Palo Alto, California), was constructed by insertion of the Tet-Off transcription unit as an XhoI to BseRI fragment. pCMVSB10 is identical to pSB10.

These plasmids were used to transform cells in the combinations shown in Fig. 8. As expected, transposition of the transposon (as determined by increased G418 resistant colony formation) was not observed in cells transfected with pTneo and ppATRES. In contrast, transposition of the transposon was observed in those cells transfected with both pTneo and ppATRESTOFF, but only in the absence of Dox (Fig. 8). These results show that a TRE-controlled transposase can regulate transposase expression and transposition in a cell.

Co-linear provision of transposon and transposase expression functions

It would be most efficient if both transposon and transposase functions could be delivered as part of the same vector in target cells and tissues. This ability was tested in a plasmid transfection experiment. pLPBLTNeo, a plasmid containing T/Neo, was constructed by digesting pT/Neo with SacI and SalI and inserting the fragment into pLPBL1. pLPBLSBTNeo, a plasmid containing the T/neo transposon and the SB transposase coding sequence linked to the CMV promoter, was constructed by as follows: a CMV-SB10 containing-fragment was obtained from pSB10 by digesting with EcoR1 and SalI and then cloning into pLPBL1 between EcoRI and SalI to generate plasmid pLPBL/CMVSB10.

T/Neo was excised from pLPBL/TNeo using XhoI and SalI and then cloned into the SalI site of pLPBL/SMVSB10 to generate pLPBLSBTNeo. pDelta28SBTNeo was constructed by excising a fragment containing SBTNeo from pLPBLSBTNeo using restriction enzyme AscI and cloning it into the AscI site of pDelta28. pDelta28TRESTONTneo wasconstructed by excising a fragment containing TRESTONTneo fragment is excised from pLPBLSBTNeo using restriction enzyme AscI and cloning it into the AscI site of pDelta28.

These plasmids were used to transfect cells in the combinations shown in Fig. 9. A 3-fold increase in the frequency of G418-resistant colony-formation was observed for the pLBPLSBTNeo construct, containing both transposon and transposase functions in cis, in comparison with pSB10 + pLBPLTNeo co-transfected cells. These results show that transposon and transposase functions can be provided by the same delivery vector, and that the frequency of transposition was in fact improved by co-delivery of transposon and transposase functions in cis.

Construction of a plasmid containing transposon, transposase, and tetracycline transactivator

The vector Ad-TRESTON/TNeo (Fig. 6b) is constructed as follows. The SB transposase coding sequences were removed from pSB10 and inserted into the multiple coning site of pTRE (Clontech). The sequence containing the TRE and the SB transposase coding sequence was excised using the restriction endonucleases XhoI and HindIII and cloned into the recipient vector pLPBL1, yielding pTRES .

An Xho1 site was engineered into position 2350 of plasmid pTet-On (Clontech) using PCR based site-directed mutagenesis. Following this, the Tet-On coding sequences were excised from pTet-On as an XhoI fragment and cloned into pTRES yielding pTRESTON.

Finally, the Neo transposon was excised from pT/NeoHSVTK with the restriction endonucleases Sal1 and Nde1. pT/NeoHSVTK was constructed by excising the HSV-tk from the plasmid pHSVTK106 (BRL, Rockville, Maryland) using Bam HI and inserted into pT/*neo*. Digestion of pT/NeoHSVTK with SalI and NdeI removed only the Neo transposon and did not remove the HSV-tk. The sites were blunted and the Neo transposon was cloned into the blunted ApaI site of pTRESTON, yielding pTRESTON/TNeo.

pTRESTON/TNeo is cleaved with a restriction endonuclease and inserted onto pΔ28, a plasmid that is packaged under the appropriate conditions to yield

the helper-dependent adenovirus vector Ad-TRESTON/TNeo (Fig. 6b)

The complete disclosures of all patents, patent applications, publications, and nucleic acid and protein database entries, including for example GenBank accession numbers and EMBL accession numbers, that are cited herein are hereby incorporated by reference as if individually incorporated. Various modifications and alterations of this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention, and it should be understood that this invention is not to be unduly limited to the illustrative embodiments set forth herein.

Sequence Listing Free Text

SEQ ID NO:1	Consensus direct repeat
SEQ ID NO:2	Direct repeat sequence
SEQ ID NO:3	Direct repeat sequence
SEQ ID NO:4	Direct repeat sequence
SEQ ID NO:5	Direct repeat sequence
SEQ ID NO:6	Direct repeat sequence
SEQ ID NO:7	Inverted repeat sequence
SEQ ID NO:8	Inverted repeat sequence
SEQ ID NO:9	Amino acid sequence of an SB transposase
SEQ ID NO:10	Nucleotide sequence encoding SEQ ID NO:9
SEQ ID NO:11	Primer
SEQ ID NO:12	Primer
SEQ ID NO:13	Primer
SEQ ID NO:14	Primer

What is claimed is:

1. A non-integrating vector comprising a polynucleotide flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a regulatory sequence comprising an operator sequence.

2. The non-integrating vector of claim 1 wherein the transposase is an SB polypeptide.

3. The non-integrating vector of claim 2 wherein the amino acid sequence of the SB polypeptide has at least about 80% identity with SEQ ID NO:9.

4. The non-integrating vector of claim 3 wherein the SB polypeptide has the amino acid sequence SEQ ID NO:9.

5. The non-integrating vector of claim 2 wherein the inverted repeats comprise SEQ ID NO:7 or SEQ ID NO:8.

6. The non-integrating vector of claim 2 wherein the inverted repeats comprise a direct repeat polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

7. The non-integrating vector of claim 1 which is a non-integrating viral vector.

8. The non-integrating vector of claim 1 which is an adenovirus-based vector comprising iterative terminal repeat sequences that flank the combination of the polynucleotide, the inverted repeats, the transposase-encoding polynucleotide, and the regulatory sequence.

9. The non-integrating vector of claim 8 that is a helper-dependent adenovirus vector.

10. The non-integrating vector of claim 1 wherein the polynucleotide flanked by the inverted repeats comprises a non-coding sequence.

11. The non-integrating vector of claim 1 wherein the regulatory sequence is a first regulatory sequence, and wherein the polynucleotide flanked by inverted repeats comprises a coding sequence operably linked to a second regulatory sequence.

12. The non-integrating vector of claim 1 wherein the operator sequence is selected from the group consisting of a *lac* operator and a tetracycline response element.

13. The non-integrating vector of claim 1 further comprising a regulatory polypeptide-encoding polynucleotide.

14. The non-integrating vector of claim 13 wherein the operator sequence comprises a *lac* operator sequence, and wherein the regulatory polypeptide-encoding polynucleotide encodes LacI.

15. The non-integrating vector of claim 13 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide encodes a polypeptide that binds to the tetracycline response element under the appropriate conditions.

16. The non-integrating vector of claim 15 wherein the regulatory polypeptide is selected from the group consisting of tTA and rtTA.

17. The non-integrating vector of claim 1 which is a plasmid vector.

18. A non-integrating vector comprising a polynucleotide flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide.

19. The non-integrating vector of claim 18 wherein the transposase is an SB polypeptide.

20. The non-integrating vector of claim 19 wherein the amino acid sequence of the SB polypeptide has at least about 80% identity with SEQ ID NO:9.

21. The non-integrating vector of claim 20 wherein the SB polypeptide has the amino acid sequence SEQ ID NO:9.

22. The non-integrating vector of claim 19 wherein the inverted repeats comprise SEQ ID NO:7 or SEQ ID NO:8.

23. The non-integrating vector of claim 19 wherein the inverted repeats comprise a direct repeat polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

24. The non-integrating vector of claim 18 which is a non-integrating viral vector.

25. The non-integrating vector of claim 18 which is an adenovirus-based vector comprising iterative terminal repeat sequences that flank the combination of the polynucleotide, the inverted repeats, the transposase-encoding polynucleotide, and the regulatory polypeptide-encoding polynucleotide.

26. The non-integrating vector of claim 25 that is a helper-dependent adenovirus vector.

27. The non-integrating vector of claim 18 wherein the transposase-encoding polynucleotide is operably linked to a regulatory sequence comprising an operating sequence.

28. The non-integrating vector of claim 27 wherein the operator sequence comprises a *lac* operator sequence, and wherein the regulatory polypeptide-encoding polynucleotide encodes LacI.

29. The non-integrating vector of claim 27 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide is selected from the group consisting of tTA and rtTA

30. The non-integrating vector of claim 18 wherein the polynucleotide flanked by the inverted repeats comprises a non-coding sequence.

31. The non-integrating vector of claim 18 wherein the polynucleotide flanked by inverted repeats comprises a coding sequence operably linked to a regulatory sequence.

32. The non-integrating vector of claim 24 which is a plasmid vector.

33. A method for making a transgenic cell comprising contacting a cell with a non-integrating vector comprising:

a selected polynucleotide flanked by inverted repeats that bind a transposase, and

a transposase-encoding polynucleotide operably linked to a regulatory sequence comprising an operator sequence,

such that the transposase is expressed in the cell to yield a transgenic cell comprising genomic DNA comprising the selected polynucleotide.

34. The method of claim 33 wherein the non-integrating vector is an adenovirus-based vector comprising iterative terminal repeat sequences that flank the combination of the polynucleotide, the inverted repeats, the transposase-encoding polynucleotide, and the regulatory sequence.

35. The method of claim 33 wherein the non-integrating vector is a plasmid vector.

36. The method of claim 33 wherein the transposase is an SB polypeptide.

37. The method of claim 33 wherein the operator sequence is a *lac* operator.

38. The method of claim 37 wherein expressing the transposase comprises maintaining the absence of a LacI polypeptide in the cell.

39. The method of claim 37 wherein expressing the transposase comprises adding IPTG to the cell.

40. The method of claim 33 wherein the non-integrating viral vector further comprises a regulatory polypeptide-encoding polynucleotide, wherein the regulatory polypeptide binds to the operator sequence under the appropriate conditions and regulates expression of the transposase-encoding polynucleotide.

41. The method of claim 40 wherein the operator sequence comprises a *lac* operator sequence, and wherein the regulatory polypeptide-encoding polynucleotide encodes LacI.

42. The method of claim 40 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide encodes tTA.

43. The method of claim 40 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide encodes rtTA.

44. The method of claim 33 wherein the polynucleotide flanked by the inverted repeats comprises a polynucleotide selected from the group consisting of a non-coding sequence and a coding sequence.

45. The method of claim 33 wherein the cell is a mammalian cell.

46. The method of claim 45 wherein the mammalian cell is a human cell.

47. The method of claim 33 performed *in vivo*.

48. The method of claim 33 performed *ex vivo*.

49. The method of claim 33 performed *in vitro*.

50. A method for making a transgenic organism comprising contacting a cell with a non-integrating vector comprising:

a selected polynucleotide flanked by inverted repeats that bind a transposase,

a transposase-encoding polynucleotide, and

a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide,

such that the transposase is expressed in the cell to yield a transgenic organism comprising genomic DNA comprising the selected polynucleotide.

51. The method of claim 50 wherein the non-integrating vector is an adenovirus-based vector comprising iterative terminal repeat sequences that flank the combination of the polynucleotide, the inverted repeats, the transposase-encoding polynucleotide, and the regulatory sequence.

52. The method of claim 50 wherein the non-integrating vector is a plasmid vector.

53. The method of claim 50 wherein the transposase is an SB polypeptide.

54. The method of claim 50 wherein the transposase-encoding polynucleotide comprises a regulatory sequence comprising an operator sequence.

55. The method of claim 54 wherein the operator sequence comprises a *lac*

operator sequence.

56. The method of claim 54 wherein the operator sequence comprises a tetracycline response element, wherein the regulatory polypeptide-encoding polynucleotide encodes tTA.

57. The method of claim 54 wherein the operator sequence comprises a tetracycline response element, wherein the regulatory polypeptide-encoding polynucleotide encodes rtTA.

58. The method of claim 50 wherein the polynucleotide flanked by the inverted repeats comprises a polynucleotide selected from the group consisting of a non-coding sequence and a coding sequence.

59. The method of claim 50 wherein the cell is a mammalian cell.

60. The method of claim 59 wherein the mammalian cell is a human cell.

61. The method of claim 50 performed *in vivo*.

62. The method of claim 50 performed *ex vivo*.

63. The method of claim 50 performed *in vitro*.

64. A method for altering the phenotype of a cell comprising:

contacting a cell with a non-integrating vector comprising

a selected polynucleotide flanked by inverted repeats that bind a transposase, and

a transposase-encoding polynucleotide operably linked to a regulatory sequence comprising an operator sequence

such that the transposase is expressed in the cell to yield a transgenic cell comprising genomic DNA comprising the polynucleotide flanked by the inverted repeats, and

determining whether a phenotype of the transgenic cell or its progeny is altered in comparison to a cell that does not comprise the selected polynucleotide.

65. A method for altering the phenotype of an organism comprising:
contacting a cell with a non-integrating vector comprising
a selected polynucleotide flanked by inverted repeats that bind a transposase,
a transposase-encoding polynucleotide, and
a regulatory polypeptide-encoding polynucleotide
such that the transposase is expressed in the cell to yield a transgenic organism comprising genomic DNA comprising the polynucleotide flanked by the inverted repeats, and
determining whether a phenotype of the transgenic organism or its progeny is altered in comparison to a cell that does not comprise the selected polynucleotide.

66. A method for making a nucleic acid delivery system comprising:
constructing a non-integrating adenovirus-based viral vector comprising
a polynucleotide flanked by inverted repeats that bind a transposase; and
a transposase-encoding polynucleotide operably linked to a regulatory sequence comprising an operator sequence; and
packaging the non-integrating adenovirus-based viral vector under conditions such that the transposase is not expressed.

67. The method of claim 66 wherein the adenovirus-based viral vector is a helper-dependent adenovirus vector.

68. The method of claim 66 wherein the operator sequence is a *lac* operator.

69. The method of claim 67 wherein packaging comprises maintaining the presence of LacI such that the LacI inhibits expression of the transposase-encoding polypeptide.

70. The method of claim 66 wherein the non-integrating viral vector further comprises a regulatory polypeptide-encoding polynucleotide encoding LacI, and wherein the operator sequence comprises a *lac* operator sequence.

71. The method of claim 66 wherein the non-integrating viral vector further comprises a regulatory polypeptide-encoding polynucleotide encoding tTA, and wherein the operator sequence comprises a tetracycline response element.

72. The method of claim 66 wherein the non-integrating viral vector further comprises a regulatory polypeptide-encoding polynucleotide encoding rtTA, and wherein the operator sequence comprises a tetracycline response element.

73. A method for making a nucleic acid delivery system comprising:
constructing a non-integrating adenovirus-based viral vector comprising a polynucleotide flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory-polypeptide encoding polynucleotide that alters expression of the transposase-encoding polynucleotide, and
packaging the non-integrating adenovirus-based viral vector under conditions that repress expression of the transposase.

74. The method of claim 73 wherein the adenovirus-based viral vector is a helper-dependent adenovirus vector.

75. The method of claim 73 wherein the non-integrating viral vector further comprises an operator sequence operably linked to the transposase-encoding polynucleotide.

76. The method of claim 75 wherein the operator sequence comprises a *lac* operator sequence, wherein the regulatory polypeptide-encoding polynucleotide encodes LacI such that the LacI inhibits expression of the

transposase-encoding polypeptide.

77. The method of claim 75 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide encodes tTA.

78. The method of claim 75 wherein the operator sequence comprises a tetracycline response element, and wherein the regulatory polypeptide-encoding polynucleotide encodes rtTA.

79. A method for treating a mammalian patient comprising administering to the patient a non-integrating viral vector comprising a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, wherein the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence is flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a second regulatory sequence comprising an operator sequence.

80. The method of claim 79 wherein the non-integrating viral vector is administered to an organ or a tissue of the patient.

81. The method of claim 80 wherein the organ or tissue is selected from the group consisting of the liver, nervous system, brain, lung, skin, cardiovascular system, heart, hematopoietic system, bone marrow, and muscle.

82. The method of claim 79 wherein the therapeutic agent is a polypeptide.

83. The method of claim 79 wherein the therapeutic agent is an RNA molecule.

84. The method of claim 79 wherein the patient has a genetic disease characterized by subnormal production of a polypeptide or RNA, and wherein the therapeutic agent comprises the polypeptide or RNA.

85. A method for treating a mammalian patient comprising administering to the patient a non-integrating viral vector comprising a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, wherein the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence is flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide.

86. A method for treating a mammalian patient comprising:
(a) explanting cells of the patient;
(b) contacting the explanted cells with a non-integrating viral vector comprising a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, wherein the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence is flanked by inverted repeats that bind a transposase, and a transposase-encoding polynucleotide operably linked to a second regulatory sequence comprising an operator sequence; and
(c) implanting the transgenic cells into the patient to cause production of the therapeutic agent in the patient.

87. The method of claim 86 wherein the cells are selected from the group consisting of hematopoietic stem cells, hepatocytes, myoblasts, fibroblasts, kerotinocytes, endothelial cells and tumor cells.

88. The method of claim 87 wherein the cells are cancerous tumor cells.

89. The method of claim 86 wherein the therapeutic agent is a polypeptide.

90. The method of claim 86 wherein the therapeutic agent is an RNA molecule.

91. The method of claim 86 wherein the patient has a genetic disease characterized by subnormal production of a polypeptide or RNA, and wherein the therapeutic agent comprises the polypeptide or RNA.

92. The method of claim 86 wherein the patient is a human.

93. A method for treating a mammalian patient comprising:

(a) explanting cells of the patient;

(b) contacting the explanted cells with a non-integrating viral vector comprising a therapeutic agent-encoding polynucleotide operably linked to a first regulatory sequence, wherein the combination of the therapeutic agent-encoding polynucleotide and the first regulatory sequence is flanked by inverted repeats that bind a transposase, a transposase-encoding polynucleotide, and a regulatory polypeptide-encoding polynucleotide that alters expression of the transposase-encoding polynucleotide; and

(c) implanting the transgenic cells into the patient to cause production of the therapeutic agent in the patient.

Fig. 1

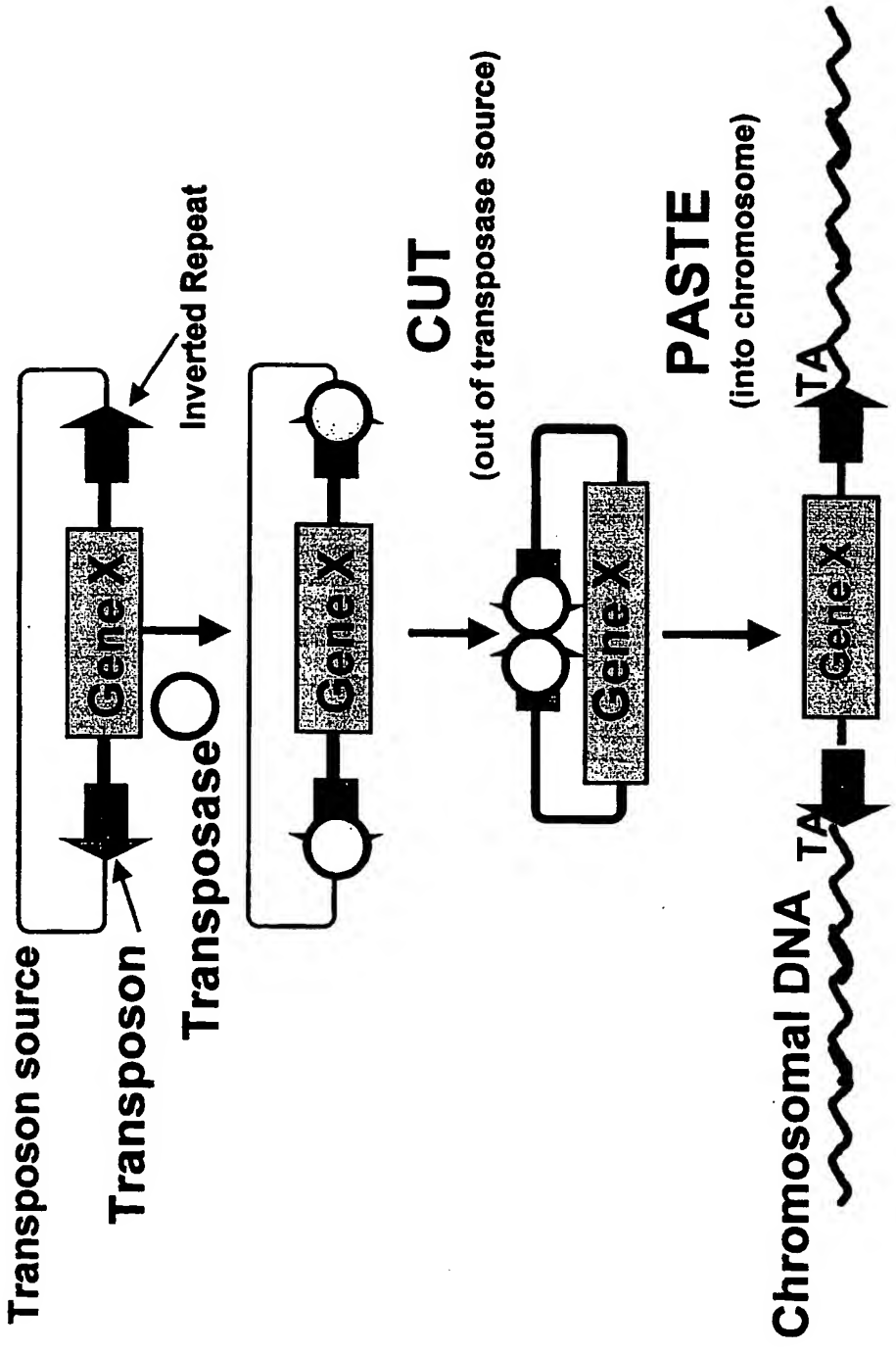

Mechanism of Transposition
Transposon source
Transposon
Inverted Repeat
Transposase
Gene X
CUT
(out of transposase source)
PASTE
(into chromosome)
TA
TA
Chromosomal DNA

Fig. 2

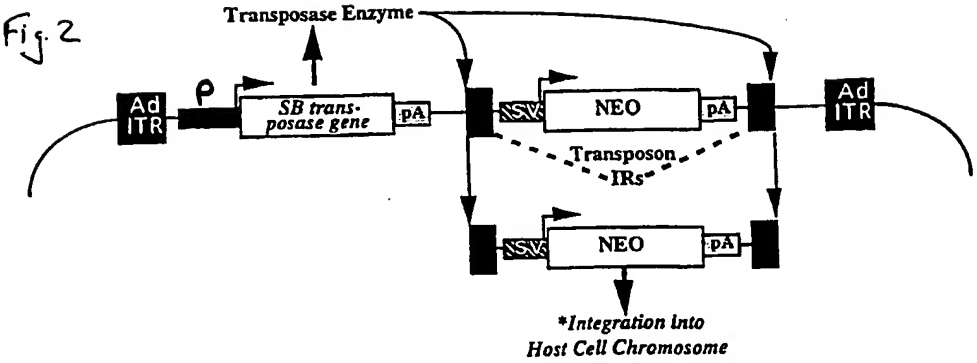
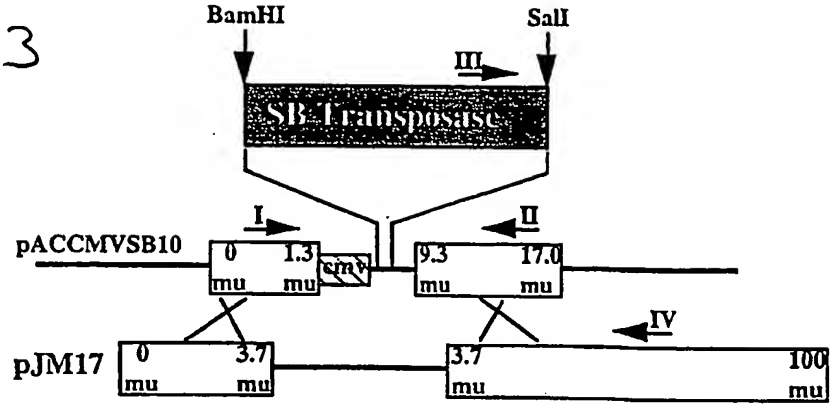

Transposase Enzyme
Ad ITR
P
SB transposase gene
pA
SSV
NEO
pA
Ad ITR
Transposon IRs
SSV
NEO
pA
*Integration into Host Cell Chromosome

Fig 3


BamHI
SalI
III
SB Transposase
I
II
pACCMVSB10
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mu
1.3
mu
cmv
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mu
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mu
3.7
mu
3.7
mu
100
mu

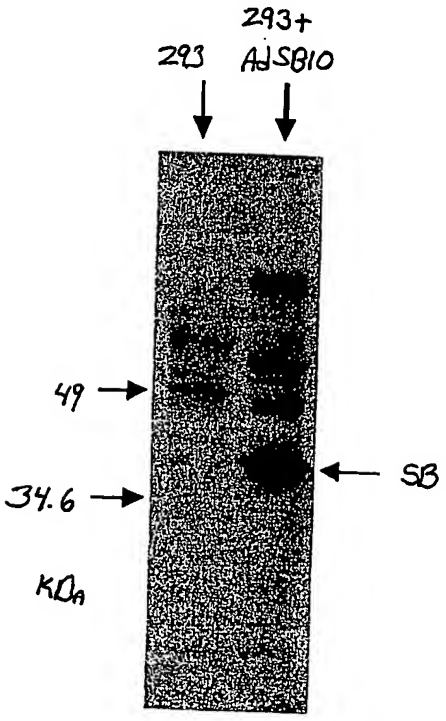

293+
293
AdSB10
49
34.6
KDa
SB

Fig. 4

Fig 5

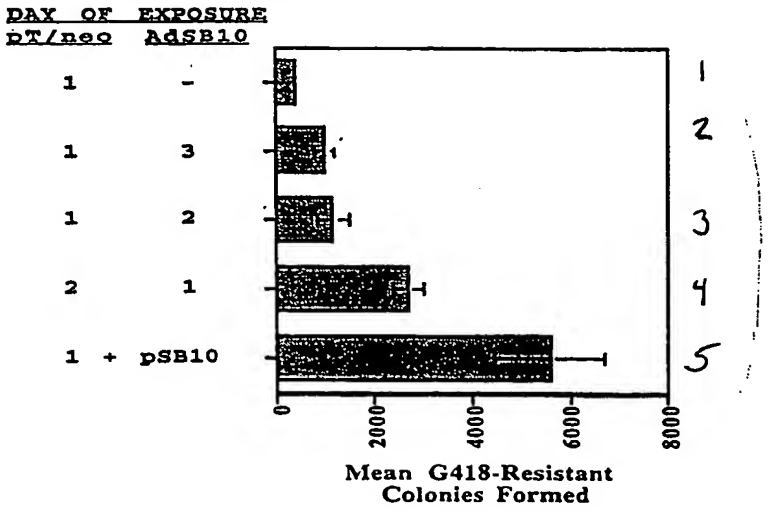
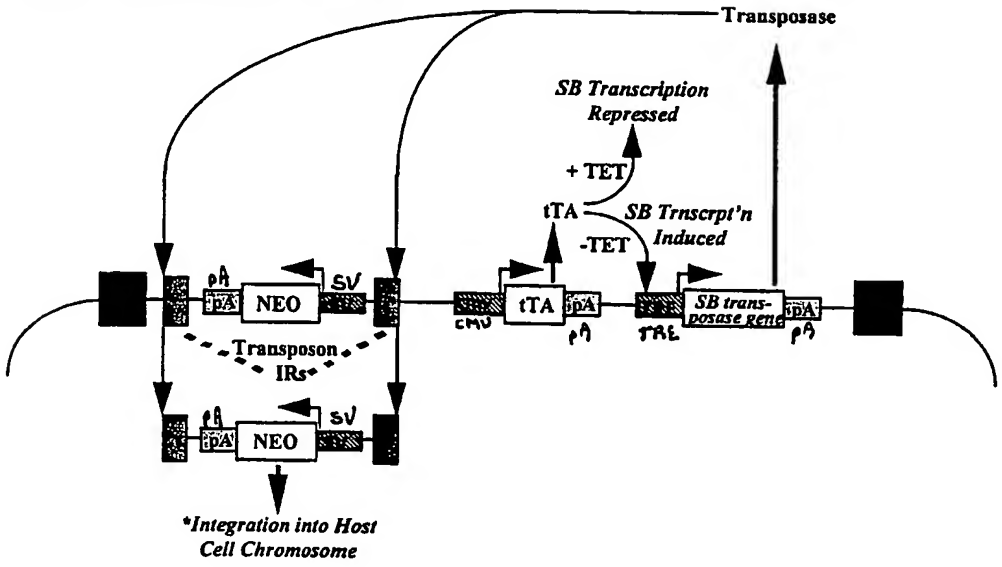

DAY OF EXPOSURE
pT/neo AdSB10
1 -
1 3
1 2
2 1
1 + pSB10
0
2000
4000
6000
8000
1
2
3
4
5
Mean G418-Resistant Colonies Formed

Fig 6

A. Ad-TRESTOFF/TNeo



B. Ad-TRESTON/TNeo

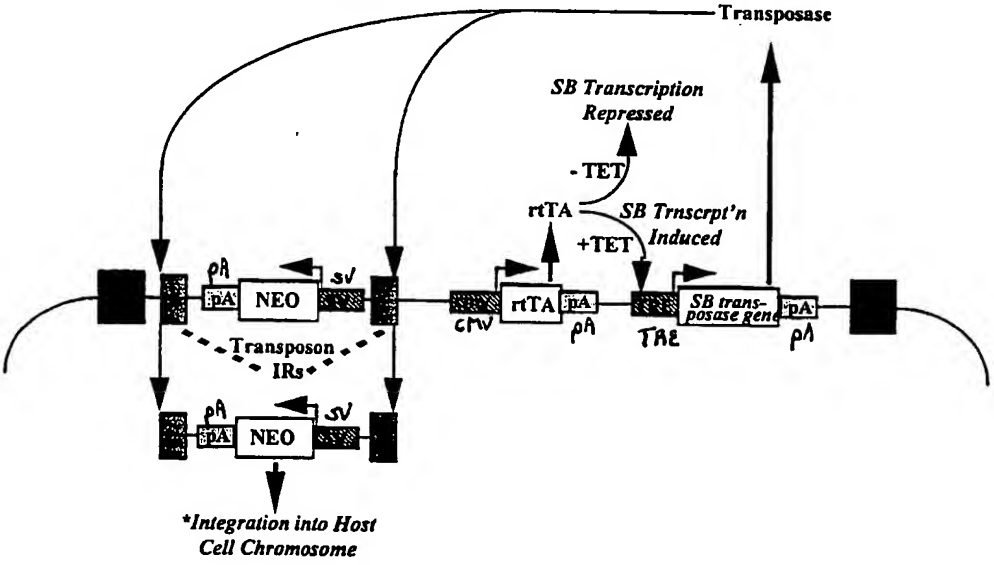
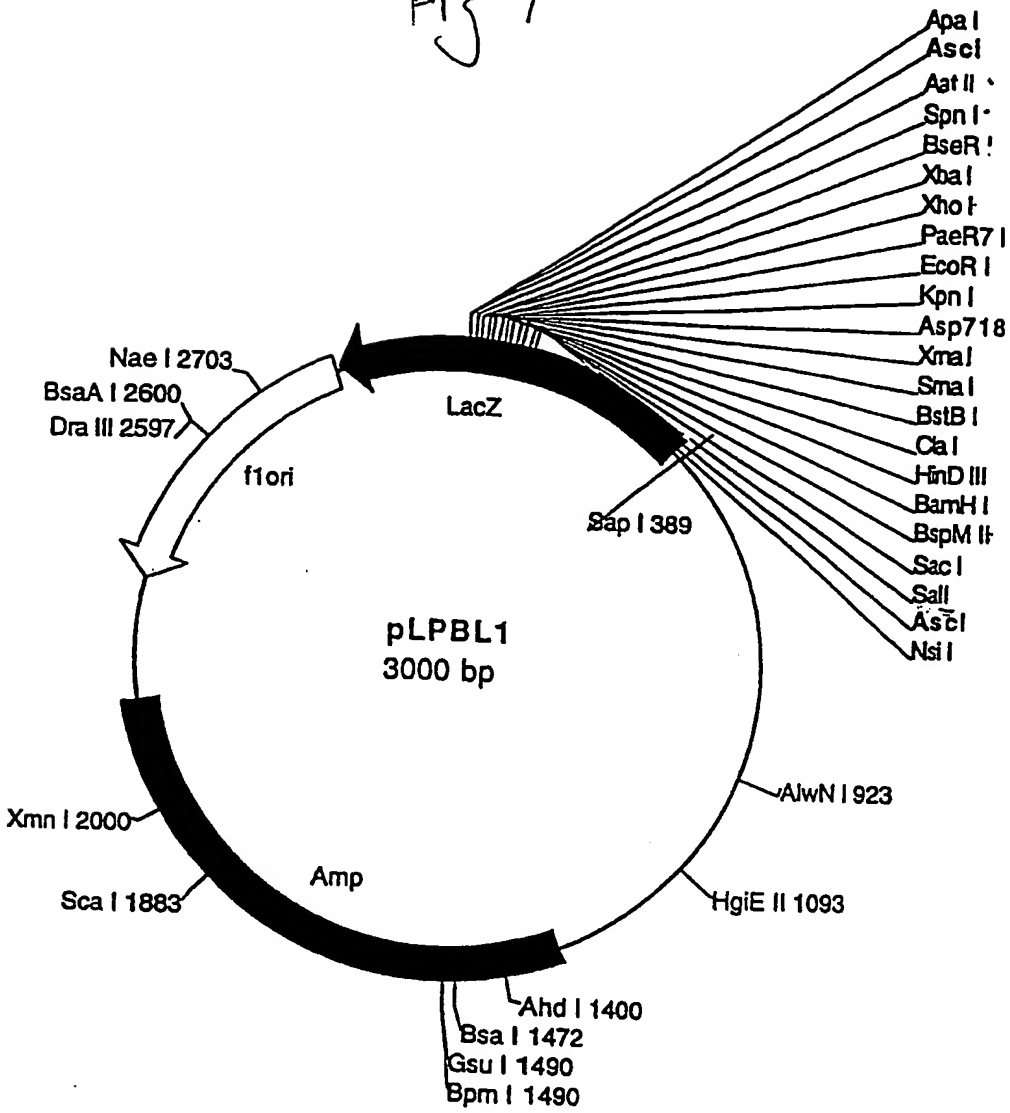


Fig 7


Apa I
Asc I
Aat II
Spn I
BseR I
Xba I
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EcoR I
Kpn I
Asp718
Xma I
Sma I
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HinD III
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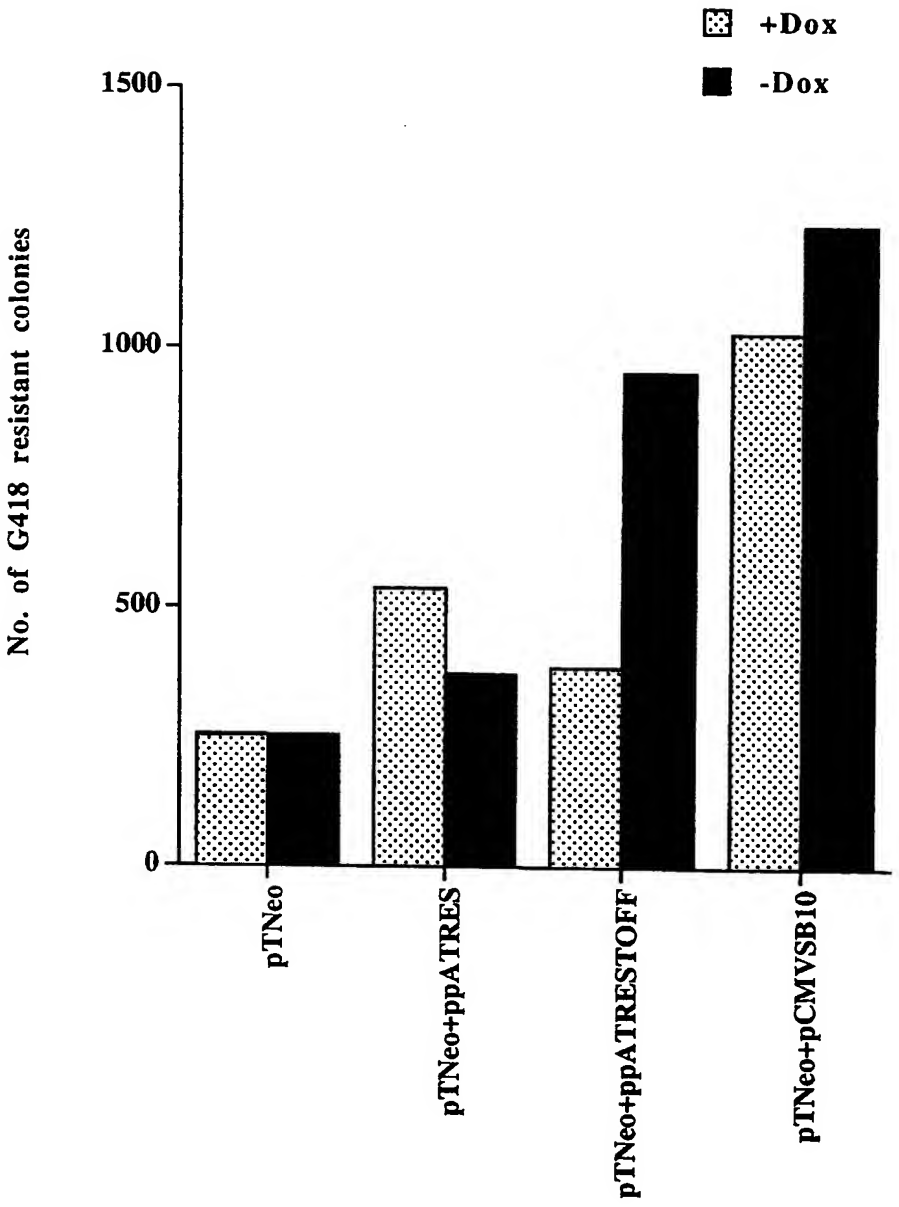
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-Dox
1500
1000
500
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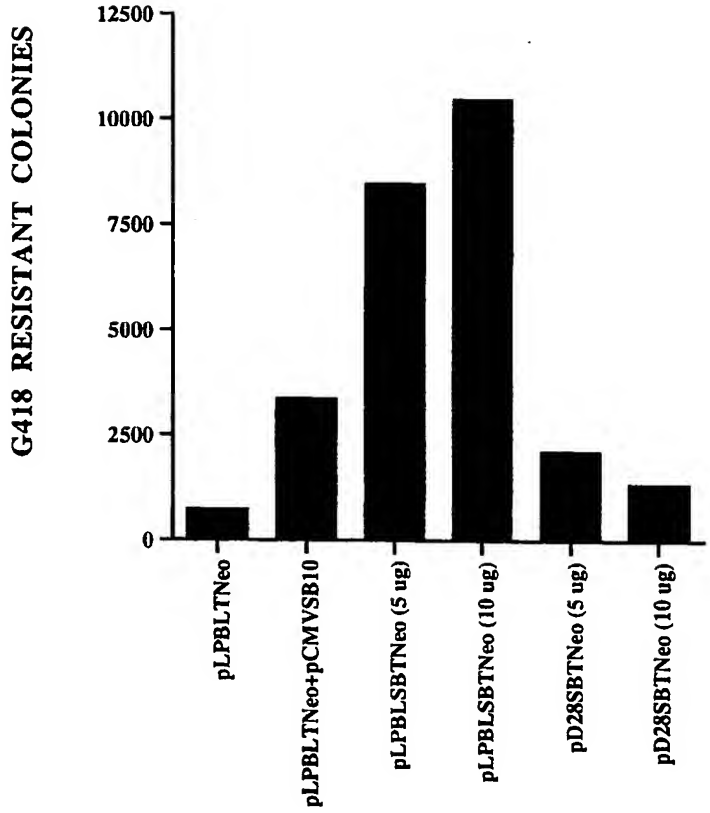
pTNeo+ppATRES
pTNeo+ppATRESTOFF
pTNeo+pCMVSB10
Plasmid transfected

Fig 8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10000
7500
5000
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pLPBLTNeo+pCMVSB10
pLPBLSBTNeo (5 ug)
pLPBLSBTNeo (10 ug)
pD28SBTNeo (5 ug)
pD28SBTNeo (10 ug)
PLASMID TRANSFECTED

Fig 9

(SEQ ID NO:3)

```
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                    TACCCTTTT AGTTTTCTTT AGTCGGTTCT GGAGTCTTTT

  51  AAAATTGTAG ACCTCCACAA GTCTGGTTCA TCCTTGGGAG CAATTTCCAA
      TTTTAACATC TGGAGGTGTT CAGACCAAGT AGGAACCCTC GTTAAAGGTT

 101  ACGCCTGAAA GTACCACGTT CATCTGTACA AACAATAGTA CGCAAGTATA
      TGCGGACTTT CATGGTGCAA GTAGACATGT TTGTTATCAT GCGTTCATAT

 151  AACACCATGG GACCACGCAG CCGTCATACC GCTCAGGAAG GAGACGCGTT
      TTGTGGTACC CTGGTGCGTC GGCAGTATGG CGAGTCCTTC CTCTGCGCAA

 201  CTGTCTCCTA GAGATGAACG TACTTTGGTG CGAAAAGTGC AAATCAATCC
      GACAGAGGAT CTCTACTTGC ATGAAACCAC GCTTTTCACG TTTAGTTAGG

 251  CAGAACAACA GCAAAGGACC TTGTGAAGAT GCTGGAGGAA ACAGGTACAA
      GTCTTGTTGT CGTTTCCTGG AACACTTCTA CGACCTCCTT TGTCCATGTT

 301  AAGTATCTAT ATCCACAGTA AAACGAGTCC TATATCGACA TAACCTGAAA
      TTCATAGATA TAGGTGTCAT TTTGCTCAGG ATATAGCTGT ATTGGACTTT

 351  GGCCGCTCAG CAAGGAAGAA GCCACTGCTC CAAAACCGAC ATAAGAAAGC
      CCGGCGAGTC GTTCCTTCTT CGGTGACGAG GTTTTGGCTG TATTCTTTCG

 401  CAGACTACGG TTTGCAACTG CACATGGGGA CAAAGATCGT ACTTTTTGGA
      GTCTGATGCC AAACGTTGAC GTGTACCCCT GTTTCTAGCA TGAAAAACCT

 451  GAAATGTCCT CTGGTCTGAT GAAACAAAAA TAGAACTGTT TGGCCATAAT
      CTTTACAGGA GACCAGACTA CTTTGTTTTT ATCTTGACAA ACCGGTATTA

 501  GACCATCGTT ATGTTTGGAG GAAGAAGGGG GAGGCTTGCA AGCCGAAGAA
      CTGGTAGCAA TACAAACCTC CTTCTTCCCC CTCCGAACGT TCGGCTTCTT

 551  CACCATCCCA ACCGTGAAGC ACGGGGGTGG CAGCATCATG TTGTGGGGGT
      GTGGTAGGGT TGGCACTTCG TGCCCCCACC GTCGTAGTAC AACACCCCCA

 601  GCTTTGCTGC AGGAGGGACT GGTGCACTTC ACAAAATAGA TGGCATCATG
      CGAAACGACG TCCTCCCTGA CCACGTGAAG TGTTTTATCT ACCGTAGTAC

 651  AGGAAGGAAA ATTATGTGGA TATATTGAAG CAACATCTCA AGACATCAGT
      TCCTTCCTTT TAATACACCT ATATAACTTC GTTGTAGAGT TCTGTAGTCA

 701  CAGGAAGTTA AAGCTTGGTC GCAAATGGGT CTTCCAAATG GACAATGACC
      GTCCTTCAAT TTCGAACCAG CGTTTACCCA GAAGGTTTAC CTGTTACTGG

 751  CCAAGCATAC TTCCAAAGTT GTGGCAAAAT GGCTTAAGGA CAACAAAGTC
      GGTTCGTATG AAGGTTTCAA CACCGTTTTA CCGAATTCCT GTTGTTTCAG

 801  AAGGTATTGG AGTGGCCATC ACAAAGCCCT GACCTCAATC CTATAGAAAA
      TTCCATAACC TCACCGGTAG TGTTTCGGGA CTGGAGTTAG GATATCTTTT

 851  TTTGTGGGCA GAACTGAAAA AGCGTGTGCG AGCAAGGAGG CCTACAAACC
      AAACACCCGT CTTGACTTTT TCGCACACGC TCGTTCCTCC GGATGTTTGG

 901  TGACTCAGTT ACACCAGCTC TGTCAGGAGG AATGGGCCAA AATTCACCCA
      ACTGAGTCAA TGTGGTCGAG ACAGTCCTCC TTACCCGGTT TTAAGTGGGT

 951  ACTTATTGTG GGAAGCTTGT GGAAGGCTAC CCGAAACGTT TGACCCAAGT
      TGAATAACAC CCTTCGAACA CCTTCCGATG GGCTTTGCAA ACTGGGTTCA

1001  TAAACAATTT AAAGGCAATG CTACCAAATA CTAG.
      ATTTGTTAAA TTTCCGTTAC GATGGTTTAT GATC.
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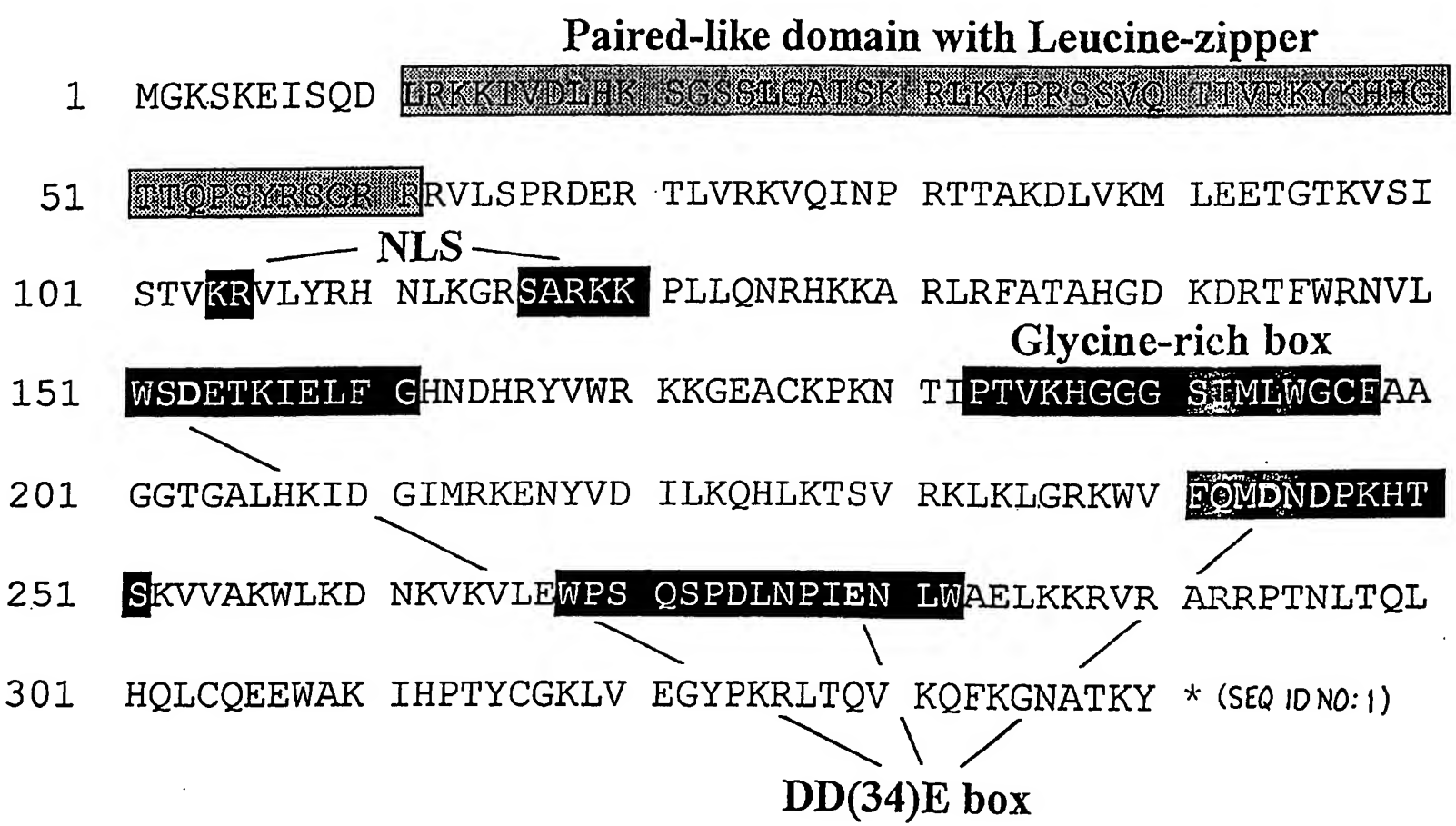
Fig. 10A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51 TTQPSYRSGR RRVLSPRDER TLVRKVQINP RTTAKDLVKM LEETGTKVSI
NLS
101 STVKRVLYRH NLKGRSARKK PLLQNRHKKA RLRFATAHGD KDRTFWRNVL
Glycine-rich box
151 WSDETKIELF GHNDHRYVWR KKGEACKPKN TIPTVKHGGG SIMLWGCFAA
201 GGTGALHKID GIMRKENYVD ILKQHLKTSV RKLKLGRKWV FQMDNDPKHT
251 SKVVAKWLKD NKVKVLEWPS QSPDLNPIEN LWAELKKRVR ARRPTNLTQL
301 HQLCQEEWAK IHPTYCGKLV EGYPKRLTQV KQFKGNATKY * (SEQ ID NO: 1)
DD(34)E box

Fig. 10B

SEQUENCE LISTING

<110> REGENTS OF THE UNIVERSITY OF MINNESOTA
BAYLOR COLLEGE OF MEDICINE
MCIVOR, R. Scott
HACKETT, Perry B.
AGUILAR-CORDOVA, Estuardo

<120> VECTOR-MEDIATED DELIVERY OF INTEGRATING TRANSPOSON SEQUENCES

<130> 110.01030201

<140> Not Assigned
<141> 2000-05-11

<150> 60/133,569
<151> 1999-05-11

<160> 14

<170> PatentIn Ver. 2.0

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<212> DNA
<213> Artificial Sequence

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<210> 3
<211> 29
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Direct Repeat Sequence

<400> 3
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<210> 4
<211> 29
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<213> Artificial Sequence

<220>
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<400> 4
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<220>
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<212> DNA
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<220>
<223> Description of Artificial Sequence: Direct Repeat Sequence

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<210> 7
<211> 226
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Inverted Repeat Sequence

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tgtgcatgac acaagtcatt tttccaacaa ttgtttacag acagattatt tcacttataa 180
ttcactgtat cacaattcca gtgggtcaga agtttacata cactaa 226

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<212> DNA
<213> Artificial Sequence

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aactgacctt aagacaggga atctttactc ggattaaatg tcaggaattg tgaaaaagtg 180
agtttaaatg tatttggcta aggtgtatgt aaacttccga cttcaactg 229

<210> 9
<211> 340
<212> PRT
<213> Artificial Sequence

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Sequence of an SB Transposase

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1 5 10 15

Asp Leu His Lys Ser Gly Ser Ser Leu Gly Ala Ile Ser Lys Arg Leu
20 25 30

Lys Val Pro Arg Ser Ser Val Gln Thr Ile Val Arg Lys Tyr Lys His
35 40 45

His Gly Thr Thr Gln Pro Ser Tyr Arg Ser Gly Arg Arg Arg Val Leu
50 55 60

Ser Pro Arg Asp Glu Arg Thr Leu Val Arg Lys Val Gln Ile Asn Pro
65 70 75 80

Arg Thr Thr Ala Lys Asp Leu Val Lys Met Leu Glu Glu Thr Gly Thr
85 90 95

Lys Val Ser Ile Ser Thr Val Lys Arg Val Leu Tyr Arg His Asn Leu
100 105 110

Lys Gly Arg Ser Ala Arg Lys Lys Pro Leu Leu Gln Asn Arg His Lys
115 120 125

Lys Ala Arg Leu Arg Phe Ala Thr Ala His Gly Asp Lys Asp Arg Thr
130 135 140

Phe Trp Arg Asn Val Leu Trp Ser Asp Glu Thr Lys Ile Glu Leu Phe
145 150 155 160

Gly His Asn Asp His Arg Tyr Val Trp Arg Lys Lys Gly Glu Ala Cys
165 170 175

Lys Pro Lys Asn Thr Ile Pro Thr Val Lys His Gly Gly Gly Ser Ile
180 185 190

Met Leu Trp Gly Cys Phe Ala Ala Gly Gly Thr Gly Ala Leu His Lys
195 200 205

Ile Asp Gly Ile Met Arg Lys Glu Asn Tyr Val Asp Ile Leu Lys Gln
210 215 220

His Leu Lys Thr Ser Val Arg Lys Leu Lys Leu Gly Arg Lys Trp Val

225 230 235 240

Phe Gln Met Asp Asn Asp Pro Lys His Thr Ser Lys Val Val Ala Lys
245 250 255

Trp Leu Lys Asp Asn Lys Val Lys Val Leu Glu Trp Pro Ser Gln Ser
260 265 270

Pro Asp Leu Asn Pro Ile Glu Asn Leu Trp Ala Glu Leu Lys Lys Arg
275 280 285

Val Arg Ala Arg Arg Pro Thr Asn Leu Thr Gln Leu His Gln Leu Cys
290 295 300

Gln Glu Glu Trp Ala Lys Ile His Pro Thr Tyr Cys Gly Lys Leu Val
305 310 315 320

Glu Gly Tyr Pro Lys Arg Leu Thr Gln Val Lys Gln Phe Lys Gly Asn
325 330 335

Ala Thr Lys Tyr
340

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<212> DNA
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acaatagtac gcaagtataa acaccatggg accacgcagc cgtcataccg ctcaggaagg 180
agacgcgttc tgtctcctag agatgaacgt actttggtgc gaaaagtgca aatcaatccc 240
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tccacagtaa aacgagtcct atatcgacat aacctgaaag gccgctcagc aaggaagaag 360
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aaagatcgta ctttttggag aaatgtcctc tggtctgatg aaacaaaaat agaactgttt 480
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caccagctct gtcaggagga atgggccaaa attcacccaa cttattgtgg gaagcttgtg 960
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tag 1023

<210> 11
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<212> DNA
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<400> 11
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<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

<400> 12
gtcacatcca gcatcacagg c 21

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<223> Description of Artificial Sequence: Primer

<400> 13
ggaaggctac ccgaaacgtt t 21

<210> 14
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 14
ccaagttgct gtccaacgcc 20

INTERNATIONAL SEARCH REPORT

	tional Application No PCT/US 00/12827

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/63 C12N15/861 C12N15/90 C12N15/55 C12N9/22 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CAB Data, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 485 701 A (AMERICAN CYANAMID CO) 20 May 1992 (1992-05-20) claims 1-12; example 1	1,7,17, 18,27, 33,37, 39,47, 50,61
Y	WO 97 15679 A (UNIV PENNSYLVANIA ;KELLEY WILLIAM MARK (US); WILSON JAMES M (US)) 1 May 1997 (1997-05-01) cited in the application the whole document	1,7,17, 18,27, 33,37, 39,47, 50,61

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 November 2000	13/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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INTERNATIONAL SEARCH REPORT

Ir. ational Application No
PCT/US 00/12827

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 29202 A (KANKER INST NL ;PLASTERK RONALD HANS ANTON (NL); VOS JAN CHRISTIAA) 14 August 1997 (1997-08-14) page 5, line 9 - line 12	1,7,17, 18,27, 33,37, 39,47, 50,61
Y	EP 0 756 007 A (AJINOMOTO KK) 29 January 1997 (1997-01-29) page 8, line 27 - line 28	1,7,17, 18,27, 33,37, 39,47, 50,61
A	WO 98 40510 A (IVICS ZOLTAN ;IZSVAK ZSUZSANNA (HU); HACKETT PERRY B (US); UNIV MI) 17 September 1998 (1998-09-17) cited in the application the whole document	
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A	WO 96 01313 A (BUJARD HERMANN ;GOSSEN MANFRED (US)) 18 January 1996 (1996-01-18) the whole document	
A	IVICS Z ET AL: "MOLECULAR RECONSTRUCTION OF SLEEPING BEATUY A TC1-LIKE TRANSPOSON FROM FISH, AND ITS TRANSPOSITION IN HUMAN CELLS" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 91, November 1997 (1997-11), pages 501-510, XP000700195 ISSN: 0092-8674 cited in the application the whole document	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/12827

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